Joana de Almeida Queiroz Menezes

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Processing data in congenital blindness: behavior and anatomy

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Supervisor: Ron Kupers, PhD, University of Copenhagen

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PROCESSING DATA IN CONGENITAL BLINDNESS: BEHAVIOR AND ANATOMY

Joana de Almeida Queiroz Menezes

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Sumário

O sistema visual ocupa mais espaço no cérebro humano do que qualquer outro sentido. Um cérebro sem visão parece sofrer alterações nas áreas corticais que originalmente participam na visão. Estudos têm demonstrado que cegos têm uma melhor *performance* que pessoas de visão normal em muitas modalidades sensoriais. Este facto é atribuído à plasticidade *crossmodal* devido ao recrutamento do córtex visual muito cedo na vida. O objetivo deste estudo é explorar como pessoas com cegueira congénita executam tarefas de discriminação e memória táctil, em relação a pessoas com visão. A experiência consistiu em ambos os grupos tocarem diferentes texturas e avaliarem as suas características qualitativas. Dez minutos após a discriminação táctil, os participantes realizaram um teste de memória táctil de curto prazo.

Surpreendentemente, os nossos resultados não mostraram qualquer diferença significativa entre as pontuações de cegos com cegueira congénita e controlos na tarefa de memória táctil de curto prazo (Sig. = 0,217) e as suas pontuações na discriminação táctil não foram significativamente diferentes em qualquer um dos cinco factores qualitativos.

Estes resultados indicam que a cegueira congénita não induz uma diferente discriminação táctil ou uma melhor memória táctil de curto prazo.

Outro estudo foi ainda feito em secções coronais de cérebro de ratinhos *WT* e *Cone-Rod Homeobox -/-* com o intuito de se registar as diferenças nas áreas S1 (córtex somatossensorial primário) e V1 (córtex visual primário), através da coloração de Nissl. Verificaram-se diferenças na espessura das camadas e nuclei em S1, mas em V1 verificou-se uma homogeneidade em ambas áreas dos dois grupos.

Palavras-chave: privação visual; plasticidade crossmodal; cegueira congénita; córtex visual; memória táctil.

Abstract

The visual system takes more space in the human brain than any other sensation. A visually deprived brain seems to undergo changes in the cortical areas originally taking part in vision and visualisation. Studies have shown that the blind outperform sighted in many sensory modalities. This has been attributed to *crossmodal plasticity* due to recruitment of the visual cortex early in life. The aim of this study is to explore how congenitally blind perform in a haptic discrimination task and a following short-term tactile memory task when compared to sighted controls. The experiment consisted of both groups touching different textures of surfaces and rate their qualitative characteristics in a quantitative rating scale. Ten minutes following haptic discrimination, the participants performed a tactile short-term memory test.
Surprisingly, our results do not show any significant difference between the scores of the congenitally blind and the sighted controls in the short-term tactile memory task (Sig. = 0.217) and their haptic discrimination scores were not significantly different in any of the five qualitative factors.
These findings indicate that congenital blindness does not induce a different haptic discrimination or better short-term tactile memory.

Another study was carried out in brain sections of WT and *Cone-Rod Homeobox* - / - mice in order to compare S1 (primary somatosensory cortex) and V1 (primary visual cortex) areas using the histological Nissl technique. There were differences in the thickness of the layers and nuclei in S1, but in V1 there was a homogeneity in both areas of the two groups.

Key words: visual deprivation; *crossmodal plasticity;* congenital blindness; visual cortex; tactile memory.

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List of abbreviations, acronyms and symbols

BLAST – Basic Local Alignment Search tool

CB – congenitally blind

CBP - CREB binding protein

Cnga3 - cyclic nucleotide gated channel alpha 3

CNS – central nervous system

CoRD – Cone-rod Dystrophy

CRX - Cone-Rod Homeobox protein

DCMLS – Dorsal Column-Medial Lemniscal System

EB – early blind

ERG – electroretinogram

fMRI - functional magnetic resonance imaging

GCN5 - histone acetyltransferase GCN5

HD - homeodomain

LB – late blind

LCA – Leber congenital Amaruosis

LGN – lateral geniculate nucleus

NCBI – National Center for Biotecnology Information

Neurod - Neurogenic differentiation

NR2E3 - Nuclear Receptor Subfamily 2 Group E Member 3

NRL - Neural retina-specific leucine zipper protein

p300 - E1A binding protein p300

PC – rapidly adapting Type II fiber

RA – rapidly adapting fiber

RA1 - rapidly adapting Type 1 fiber

RNA - ribonucleic acid

RP – Retinitis pigmentosa

rTMS – repetitive Transcranial Magnetic Stimulation

S1 – primary somatosensorial cortex

SA1 – slowly adapting Type 1 fiber

SAII - slowly adapting Type II fiber

SC – blindfolded sighted control

V1 – primary visual cortex

VPL - ventroposterial lateral nucleus

VPM - ventroposterior medial nucleus

WT – wild type

Introduction

1.1. Touch

1.1.1. An object of study

In our daily basis, in a continuous way, we explore surfaces with our hands and fingertips. We use the sense of touch. The major responsible organ for this sense is the biggest one, the skin. The hands make it possible for us to sense, manipulate and discriminate between dangers and pleasantness around us. Through the hands we can perceive different objects and their physical state, e.g. liquid, solid or gaseous state. In addition to this we sense other characteristics such as dimensions, shape, aspects and many others. Without sight, touch is our main navigation to the world and is therefore of great interest as an object of study in the field of compensatory adjustment in the congenitally blind. Once learning to read Braille, blind find great relief in gaining more information that they otherwise lack, because of their blindness.

1.1.2. Mechanoreceptors in the human hand

The skin is a large flexible organ that is deformed when in contact with an external stimulus. The deformation results in activation of specific receptors, mechanoreceptors, that allows us to perceive touch (Gardner, 2010). Depending on the spatial localization, glabrous or hairy skin, receptive field, types of mechanoreceptors activated and which combination of activation we are able to detect different characteristics of objects, namely their size, shape, their texture if it is smooth, rough, cold or warm in temperature and if it is pleasant to touch or not (Gardner, 2010).

1.1.3. Types of mechanoreceptors

There are four types of mechanoreceptors in the hand: Meissner corpuscle, Pacinian corpuscle, Merkel neurite complex and SAII end organ and they can be distinguish based in two characteristics, such as: their rate to skin adaptation and on the size of their receptive fields (Bolanowski *et al*, 1988; Gescheider *et al*, 2009; Obrist *et al*, 2013). The Meissner corpuscle and Merkel neurite complex are considered the principal touch receptors in the glabrous skin, such as the palm, fingers and sole of the foot (Gardner, 2010). These receptors are located in the surface

of the skin, namely in the papillary ridges, forming this way the fingerprints and they will also allow us to feel the Braille dots and discriminate the different textures with a great acuity (Gardner, 2010). The human hands have approximately 150.000 mechanoreceptors with round receptive fields of different sizes connected in a dense network that interacts when stimulated. They are connected to the CNS through 30.000 primary afferent fibers of the Aβ type with great conduction velocity (Gardner, 2010) making it possible to activate withdrawal reflexes and get instant conscious and unconscious information about touch. Each fingertip is innervated by around 250-300 mechanoreceptive fibers, making it one of the areas with the highest density of receptors, 2500 per cm^2 (Gardner, 2010) and the hands one of the most sensitive areas to touch in humans, just as shown in the homunculus of the primary somatosensory cortex, SI (Snyder & Whitaker, 2013).

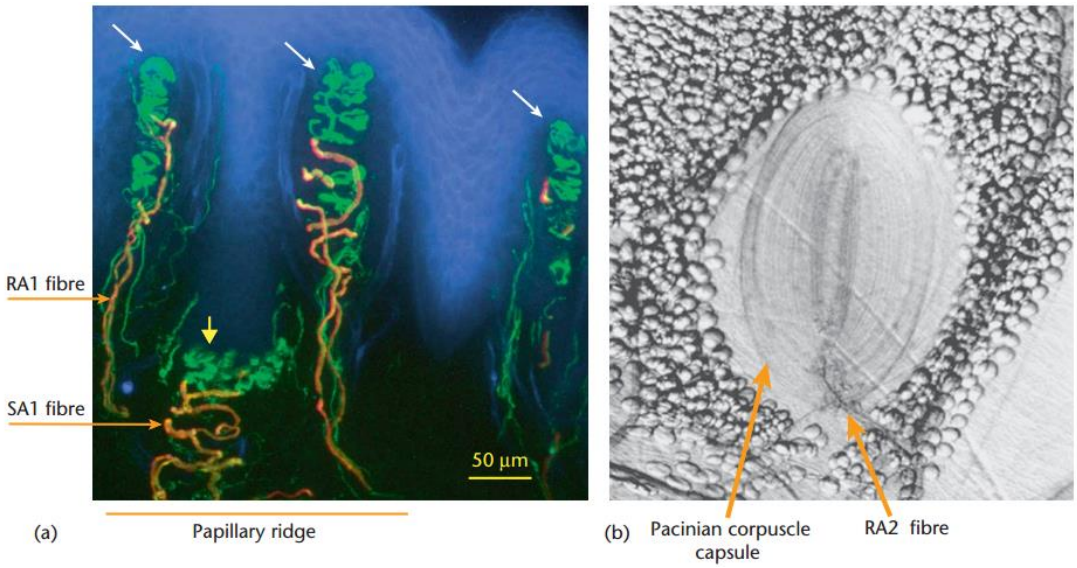

Fig.1.1. Mechanoreceptors in the skin. (a) Image from a papillary ridge (fingerprint) of Meissner corpuscles (white arrows) and Merkel cells (yellow arrow) by confocal microscopy innervated by their respective fibers: RA1 and SA1. (b) Pacinian corpuscle located in the mesentery of the abdominal wall, innervated by only one RA2 fiber. Adapted from Gardner, 2010.

The rate of adaptation of the nerve fibers or cutaneous tactile sensors in the fingertips is named slowly adapting and fast/rapidly adapting receptors (Jamali & Sammut, 2010; Obrist *et al*, 2013) and they will have an important role in the sensory feedback when handle with different objects and materials because according to Abraira and Ginty (2013) receptors are qualitatively different and they will be activated according to specific tactile sensation (Kandel *et al*, 2000). Thereby they are part of the somatosensory system and they are located in the glabrous skin, e.g. the sole of the feet or the palm of the hands (Obrist *et al*, 2013).

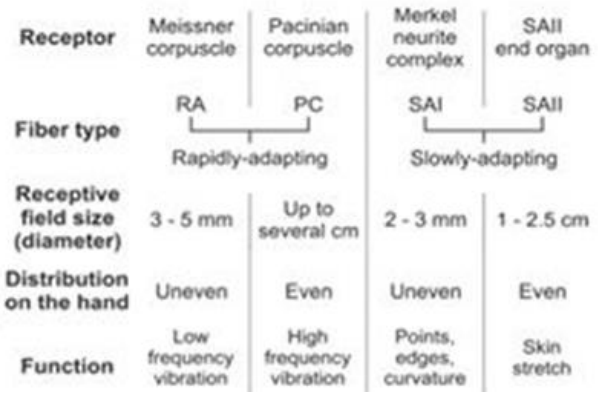

Fig.1.2. The mechanoreceptors characteristics: fiber types, receptive field size, distribution on the hand and function. Adapted from Obrist *et al*, 2013.

The slowly adapting mechanoreceptors are responsible for the static characteristics of a stimulus so they will be active when there is a continuous stimulus (Kandel *et al*, 2000). These mechanoreceptors are Merkel neurite complex and SAII end organ.

Contrary to the ones referred to above, Meissner- and Pacinian corpuscles are fast adapting mechanoreceptors that will propagate action potentials in the beginning and in the end of a stimulus (Kandel *et al*, 2000). Only Meissner- and Pacinian corpuscles respond to vibration (Obrist *et al*, 2013). Meissner corpuscle responds to vibrations ranging from 2 to 40 Hz frequency while Pacinian corpuscles respond to vibrations of frequencies more than 40 Hz in a U-shaped response curve contrary to the first ones which response curve is characterized as flat (Obris *et al*, 2013) so depending on the frequency the sensation will stimulate a specific type of receptor. Regarding the other mechanoreceptors, Merkel neurite complex and SAII end organ, they will respond to other types of external stimulus: points, edges, curvature and skin stretch (Obris *et al*, 2013).

Receptive fields of Pacinian corpuscle and SAII end organ are large and can cover a whole fingertip or even a hand compared to Meissner corpuscle and Merkel neurite complex, which receptive fields are smaller and more defined (Obrist *et al*, 2013). Pacinian corpuscle and SAII end organ are mechanoreceptors better distributed in the hand while Meissner corpuscle and Merkel neurite complex are more concentrated in the fingertips and start to be less dense from there till the palm of the hand (Obrist *et al*, 2013).

1.1.4. The sensation of touch

When being touched, mechanoreceptors respond to the pressure or stretch of the surrounding tissue by opening cation channels and transduce the deformation into electrical energy. When the receptor is relieved of the mechanical stress, stretch-sensitive channels close and it stops firing

action potentials (Arendt-Nielsen & Chen 2003; Franç Ois et al. 2015; Leem et al. 1993; Mouraux et al. 2003). Depending on the mechanoreceptors activation, the stimuli gives the individual information about the qualities of the material (Klöcker 2014).

Sensations can be divided into four attributes, such as modality, location, intensity and time course (Kandel *et al.* 2000). The modality depends on the type of energy transmitted and receptor types that responds. In regards to the location, different receptors will be activated when they respond to stimuli with their different sized fields (Kandel *et al*, 2000). The intensity depends on the quantity of energy that is delivered to the receptors and the timing when the response by the mechanoreceptors starts and stops. The resulting patterns of activation activates sensory neurons with corresponding action potentials (Kandel *et al*, 2000). In figure 1.7. the spike trains represent the action potentials.

The external stimulus and the sensation will lead to a skin deformation (Gardner, 2010; Obrist *et al*, 2013) and will be transformed into an electric signal, so it will be transduced by the mechanoreceptors (Gardner, 2010). Later this information is translated into an action potential and sent to the brain by the peripheral nerves and consequently will give us information about the size, shape, among others characteristics of the object (Obrist *et al*, 2013).

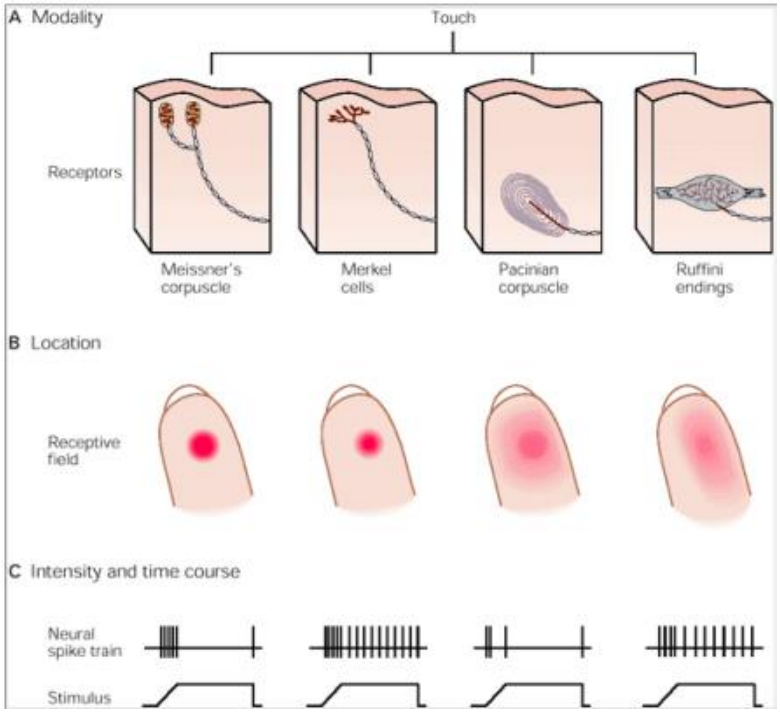

Fig.1.3. The four attributes of a sensation. (A) modality, (B) location and (C) intensity and time course.

1.1.5. The primary somatosensory cortex

Spatial acuity of touch depends on the density cutaneous mechanoreceptors (thereby small receptive fields) and is greatest on the fingertips and lips where the receptors are most abundant and the receptive fields smallest (roughly seen on Penfield's Homunculus of the somatotopic arrangement of the somatosensory inputs of the human cerebral cortex (Snyder & Whitaker 2013)).

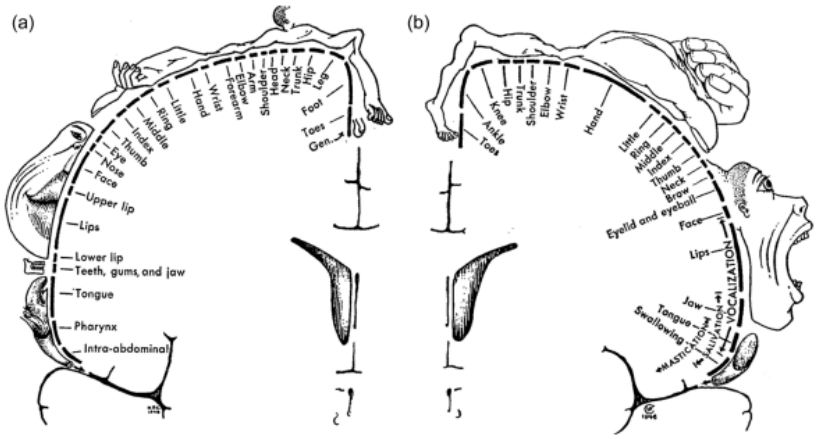

Fig. 1.4. The (a) sensory and (b) motor homunculi with their figure captions (adapted from Snyder & Whitaker 2013; printed in Penfield & Rasmussen, 1950).

The cutaneus mechanosensitive afferent nerve fibers in the epidermis and dermis belong to the Aβ nerve fibers that are thick, highly myelinated and thereby have a high conduction velocity. Aβ fibers convey touch through pseudounipolar cells to the spinal cord to the Dorsal Column-Medial Lemniscal System (DCMLS), which is the principle pathway for touch and proprioception (Kandel et al. 2000). From the DCMLS in the medulla spinalis, the action potential passes to the gracile or cuneate nuclei to the primary somatosensory cortex.

The primary somatosensory cortex or S1 is located in the lateral postcentral gyrus, in the parietal lobe. It receives the somatotopic input from the thalamus, namely from the ventroposterial lateral nucleus (VPL) and ventroposterior medial (VPM) thalamic nucleus. Moreover S1 is considered the sensory receptive area for the sense of touch, from the toe till the head and therefore will be divided in different neural points that will correspond to the position of a receptor in a certain part of the body. In contrast, a light touch, cold, heat or a painful stimuli in the same areas of the body will have different pathways to the brain (Kandel *et al*, 2000).

1.2. The brain.

1.2.1. The primary visual cortex

The cerebral cortex is a thin layer, rich in neurons, that is responsible for our adaptive response to outside stimuli from the world, namely audition, somesthesis, vision, movement, among others. The human cerebral cortex is the most convoluted of all vertebrates and has a very characteristic shape with *sulci* that is an evolutionary adaption that allows the cortical area to be much bigger and have a superior number of neurons, which in turn increases cortical information processing without the brain having a bigger volume.

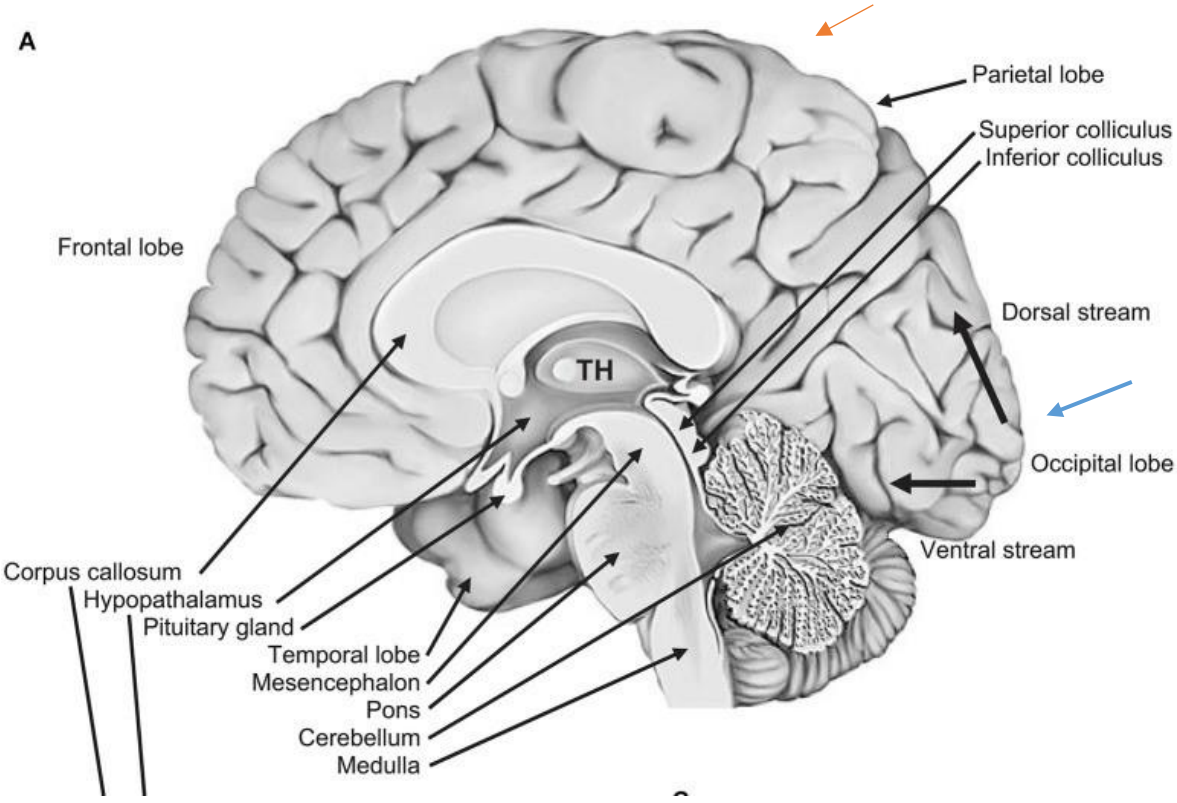

Fig.1.5. (A) Medial view of the brain where it can be seen the different lobes, with special emphasis to the occipital lobe where the visual cortex is located (blue arrow). Coubard, O.A. *et al*, 2014. Educating the blind brain: a panorama of neural bases of vision and of training programs in organic neurovisual deficits. Front. Integr. Neurosci. 8:89. (© O.A. Coubard, with permission).

The cerebral cortex of humans and mice can be divided into 6 layers as described by Brodmann in 1908 and 1909, each layer differentiated by constitution, e.g. difference in cells type and absence or prominence of neuronal cell bodies. The visual cortex layer IV can be further subdivided in 3 layers and is very prominent compared to other layers due to the large inputs from thalamus, especially in humans, thereby receiving large amounts of sensory information from the bilateral nuclei located in the inferior surface of the thalamus, through white matter fibers, called the lateral geniculate nuclei (LGN).

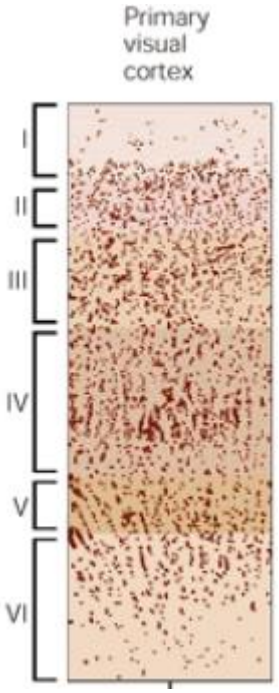

Fig. 1.6. The layers I, II, III, IV, V and VI of V1. It is observable the different prominence in the cell layers. Adapted from Kandel *et al*, 2000.

The cerebral cortex can be anatomically divided in 5 lobes: insula, parietal, occipital, temporal and frontal. Each lobe can be divided into areas of specific cognitive functions, sensory modality or motor function, consequently making the areas responsible for different processes of information. The primary visual cortex is located in the occipital area as it can be seen below on Fig.1.3 and it surrounds the calcarine sulcus. Its main function is to receive the visual input from the eyes through the optic chiasm, to LGN and to the primary visual cortex.

The visual cortex is dedicated to interpret stimuli from the eye to give a conscious perception of light sources around us. In the blind it is unclear how much of this function remains. The visual cortex comprises of a large part of the cerebral cortex, thereby making it huge sensor for information processing.

1.3. The blind brain

1.3.1. The vision and congenital blindness as a study object

The retina of the eyes contains millions of bipolar photoreceptors that allows light of different wavelengths to be converted from photons into action potentials that are transmitted to ganglion cells, the optic nerve and further to the CNS (lateral geniculate body, superior colliculus, suprachiasmatic nucleus, the nuclei of the optic tract, optic radiations, primary visual cortex and visual associated cortex). Vision is the most dominating sense of the human cerebrum and is of critical importance in understanding our surroundings and adapting to the world around us.

Without visual perception some daily basis tasks can be truly demanding. Loosing vision can lead to major disability and over time lead to striking adaptations in the human brain.

The visual cortex responsible for the visual processes is about one-third of the all cortical area in humans and non-humans primates (Kupers, 2011a) which means that studying the visual system of visually deprived brains makes it possible to understand and analyse the cerebral changes that occur when such an important sense is lost, namely on sensory and motor cortical maps due to inactivation of the afferent or efferent pathways during early development (Buonomano & Merzenich, 1998). Congenitally blind are in the rare situation that they have been without a functional visual system since birth. Thus, the studying of congenitally blind individuals gives us a unique chance to gain insight into the processes that occur in the brain regarding its re-organization and compensatory behavioral changes that take place as a result of sensory deprivation, i.e. processes of compensatory plasticity.

Compared to normal sighted people, blind people have a greater activation of the visual brain areas during non-visual tasks, e.g. tactile and auditory tasks (Wanet-Defalque *et al*, 1988; Uhl *et al*, 1991; Uhl *et al*, 1992; Rösler *et al*, 1993; Sadato *et al*, 1996; Röder *et al*, 1997; Büchel *et al*, 1998; Sadato *et al*, 1998; De Volder *et al*, 1999; Théoret *et al*, 2004; Wong *et al*, 2011). Furthermore congenitally blind individuals have been shown to be remarkably superior to sighted peers in serial memory tasks and slightly better in item memory tasks (Raz *et al*, 2007). The substantial evidence that congenitally blind individuals outperform normal sighted controls in a variety of non-visual tasks indicate that blind individuals develop superior sensory abilities, possibly in order to compensate for their lack of vision.

1.3.2. *Crossmodal plasticity* and the blind

Normal sighted fingertip accuracy has been shown to be better in Braille reading than untrained sighted controls after merely having five days of complete visual deprivation (Kauffman *et al*, 2002). This is an example of fast cortical re-organization of the visual cortex due to visual deprivation in adults that gives a better understanding of why visual brain areas are active after sensory deprivation, namely during non-visual tasks (Théoret *et al*, 2004).

A review by Rauschecker, in 1995, found that a visual deprived or injured brain is able to reorganize itself due to the practice of non-visual abilities. This compensation ability can be considered a survival adaptation that will increase our need to have information about our surroundings, inactivating skills of no stimuli and enhancing others. This has become a fascinating field of study with great rehabilitation prospects for those afflicted with sensory deprivation. This phenomenon is called *crossmodal plasticity*.

Congenitally blind people have at birth or just after birth lost one of the most important sources of information for human beings, sight. The visual system is the biggest part of our cortex and it is therefore very interesting to gain insight and better understand the cerebral reorganization in a group like the congenitally blind where the cortical areas do not receive their normal sensory input.

There is an expansion of the sensory-motor cortical representation of the reading finger (right index), compared with the left index, in the blind (Pascual-Leone and Torres, 1993, *in* Goldreich and Kanics, 2003), causing them to have higher fingertip acuity than the sighted (Alary *et al*, 2009).. Therefore we could expect the blind to have larger, better defined and denser somatosensory cortical areas, especially for those who read Braille. According to studies they learn Braille by activating V1 when performing these tasks (Wittenberg *et al*, 2004). In this way they recruit the occipital cortex for somatosensory tasks or they do indeed improve visualisation with another afferents sensation than sight. Either way it can be attributed to the phenomenon of *crossmodal plasticity.*

One of the most used senses to compensate for the loss of vision in the blind is the tactile sense, e.g. for Braille reading. This could hypothetically induce *crossmodal plasticity* and it is therefore interesting to explore the haptic sensation and tactile acuity of the blind.

1.3.3. Congenital blindness vs Normal vision

1.3.3.1. What is congenital blindness?

1.3.3.1.1. Definition of congenital blindness

Congenital blindness can affect different life factors, such as infant development, education, social- and marital future and economic prospects (Gogate *et al*, 2011).

Congenital blindness is characterized by individuals that had never received visual input in their lives and that have been blind since birth. *Early blind* groups can include congenitally blind subjects. The early blind are considered the cases that occur in the first years of life up to 5 years old. *Late blindness* is characterized for being blind after puberty or in their adulthood (Voss, 2013).

Subjects with an early onset blindness show much more *crossmodal plastic* changes compared to late onset blind (Voss, 2013). There is thereby not only a critical period for sight with visual deprivation, but also a critical period for *crossmodal plasticity*, after which it becomes more difficult for the blind brain to compensate for the loss of vision through enhancement of other senses by recruiting the deprived areas.

1.3.3.2. Etiology of congenital blindness

Hubel and Wiesel (1964) experimented with afflicting blindness early in life, trying to immolate congenital blindness by suturing eyelids of kittens. No retinal cell or lateral geniculate nuclei changes happened, but they turned blind anyway. In children congenital cataract or uncorrected strabismus/amblyopia before the age of 3 leads to loss of vision to the affected eye (Sjølie *et al*, 2013). These findings heavily suggest that sight has to develop within critical period of time to function properly or turn blind.

Congenital blindness is thereby not only blindness from birth, but is caused by birth defects or deprivation in the visual system in early life, causing blindness to the young child. Some of the known causes are anophtalmoia, microphtalmos, coloboma, congenital cataract and retinal dystrophies, such as Leber's congenital amaurosis, infantile glaucoma and congenital cloudy cornea (Gogate *et al*, 2011).

1.3.3.3. Studies on congenitally blind and sighted subjects

In this section, selected literature will be presented in order to review the current understanding of *crossmodal plasticity* and the sensory modalities tested in congenitally blind individuals.

Literature reviewed on tactile, auditory, gustatory and olfactory processing in blindness (Kupers and Ptito, 2014) shows that visual deprivation re-organises the visual cortical areas, gaining new functions instead of neural cell-death or inactivation. Congenitally blind subjects activate not only the olfactory brain areas but also their occipital cortex in an easy odor detection (Kupers *et al*, 2011b). The brain thereby shown adaptations to the environment (Kupers, 2011a; Ptito *et al*, 2012) meaning that the brain is pliable (Ptito *et al*, 2012) and will change and mold continuously following sensory deprivation, brain injury or abnormal development, termed *training dependent plasticity*, part of the *crossmodal plasticity* (Kupers, 2011a).

The blind have been tested in a series of different sensory modalities comparing them to sighted. Congenitally blind showed a lower threshold for activation of nociceptors C and Aδ, which means that their temperature discrimination is enhanced compared with sighted people and can be due to changes in the cortical area (Slimani *et al*, 2015).

Studies exploring tactile and haptic sensations also show that blind subjects outperform the sighted subjects in these non-visual tasks as it can be seen in the studies done by Goldreich and Kanics, in 2003 and Alary *et al*, in 2009. In Goldreich and Kanics, 2003 the volunteers did an automated grating orientation task that showed that passive tactile acuity was greater in the blind, so there is evidence that loss of sight leads to tactile acuity enhancement instead of the tactile

experience. Alary *et al*, 2009 corroborates Goldreich and Kanics, 2003 in that blind subjects were better in a texture discrimination task, meaning that they enhanced their tactile acuity compared with the sighted subjects that can be explained by activation of the visual cortex due to cortical plasticity.

When testing Braille reading, it activated the visual cortex in CB, EB and LB subjects in studies performed by Pons in 1996 and Sadato *et al* in 1998. In 2001 Burton *et al* confirmed these results in a study using fMRI. Tested during Braille reading (*active touch*) and where an experimenter helped participants touch random dot patterns with their fingertips (*passive touch*) even showed that glucose metabolism in the inferior occipital lobe were greater in the congenitally blind compared with the sighted controls (Fig.1.2) and they had superior cerebellar flow (Uhl *et al*, 1993) that could be attributed to *crossmodal plasticity*.

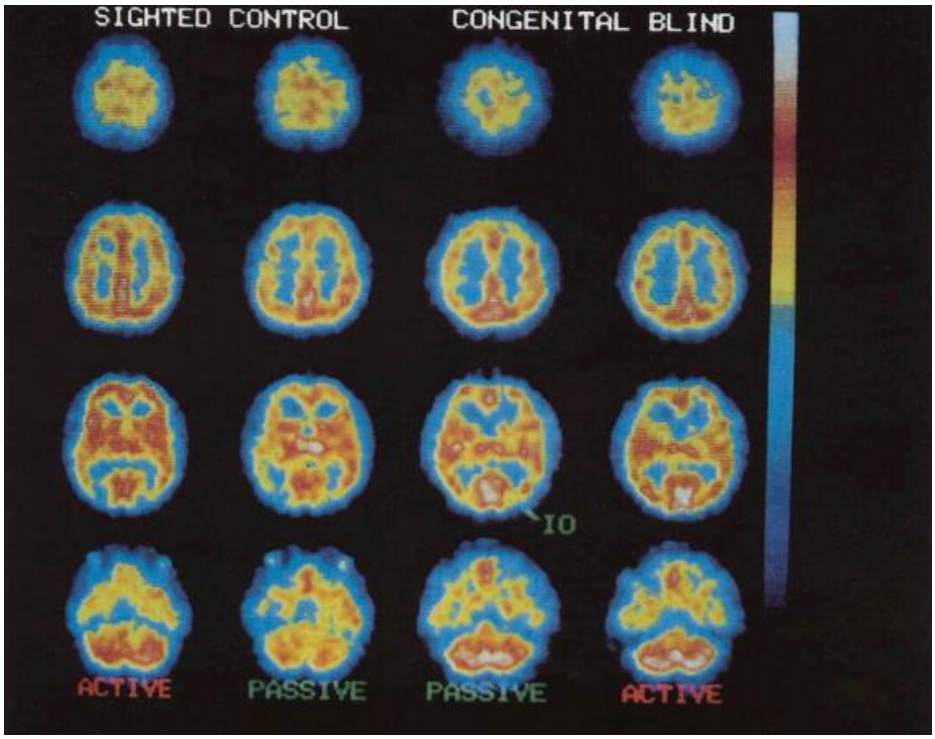

Fig.1.7. Increased regional cerebral blood flow in inferior occipital cortex and cerebellum of early blind and sighted control persons, measured by Brain-SPECT. To the left: right hemisphere. To the right: left hemisphere. Inferiorly: postcentral part of the brain and cerebellum. Superiorly: Precentral part of the brain. Adapted from Uhl *et al*, 1993.

New revelations in the field of *crossmodal plasticity* has been possible due to brain imaging studies including fMRI and rTMS. These techniques have allowed us to more closely studying the direct neuroanatomical consequences of sight deprivation. Results from Kupers (2011a) provides evidence that the occipital cortex of congenitally blind subjects is activated by a variety of non-visual tasks, including tactile discrimination. E.g. Braille reading activated the visual

cortex in CB, EB and LB subjects in studies performed by Pons in 1996 and Sadato *et al* in 1998. Results confirmed by Burton *et al*, using fMRI in 2001.

The sensory modalities tests in the blind has the undelying paradigm that brain areas related to visual processing can be recruited through compensatory cross-modality, so these visual cortical areas can be recruited for non-visual tasks (Iversen *et al*, 2015). *Crossmodal plasticity* explains why loss of vision from birth, early or even later in life makes major alterations in the organization of the relevant brain areas that are classically thought to contribute to cognitive tasks in neuroanatomy through stimulation of neural connections, enhancing the remaining senses by recruiting the visually deprived cortex of congenitally and early blind people to other functions.
In the following section we will have an inside look at the molecular genetics that support the *crossmodal plasticity* paradigm.

1.4. *The Crx brain*

1.4.1. Gene definition

The *Crx brain* creates a great opportunity to study congenital blindness and neuroplasticity through molecular genetics due to the great homogeneity between the gene in mice and men, making it possible to extrapolate an animal study.
CRX stands for cone-rod homeobox and it is an otd/Otx-like homeodomain transcription factor that is mainly expressed in cones and rod photoreceptors in the retina, playing an essential role in the differentiation of these cells (Chen *et al*, 2002; Furukawa *et al*, 1997) and are fundamental in their transcription regulation (M.Tran *et al*, 2014).
Crx encodes a protein with 299 amino acids that is much preserved in mammals (Chen *et al*, 2002). It will be very important for the survival of the photoreceptors (Furukawa *et al*, 1997).
CRX contains an otd/Otx-like paired HD that is located near the N terminal, next to glutamine rich (Gln), basic, WSP and Otx-tail domains (Chen *et al*, 2002), while the C-terminal region of CRX is necessary to the transactivation activity (Chau *et al*, 2000). CRX proteins are expressed principally by the photoreceptors of the retina and pinealocytes of the pineal gland in the adult (Chen *et al*, 1997) and binds and activates different promoters of photoreceptor-specific genes, e.g. rhodopsin, PDE, among others (Chen *et al*, 1997, in Chen *et al*, 2002). In the mouse, CRX are expressed in the retina of the embryon on day 12,5 (Chen *et al*, 1997), which means that is very important for its normal development.
Moreover CRX interacts with NRL, NR2E3, and will be co-activator for GCN5, CBP and p300, which means that it has a big role in controlling photoreceptor expression (M.*Tran*, 2014).

1.4.2. *Crx* mutants

In humans CRX mutations result in dominant retinopathies, such as RP, CoRD and LCA that are related to photoreceptor degeneration that leads to loss of vision (Kusuwama *et al*, 1997). In order to study the genetic principles of *Crx* in human diseases, *we can use mouse studies to extrapolate to a human population, due to the fact that the sequences of Crx are* homologous (Pearson, 2013) and are 97% identical (BLAST on NCBI) to human Crx.
In the homozygous *Crx Knock-Out* mouse ("-/-") the photoreceptors are dysfunctional (M.Tran *et al*, 2014) and they don't have outer segment structures (Furukawa *et al*, 1999). Moreover assayed in an ERG there won't be cone and rod activity in these mice (Furukawa *et al*, 1999).

Heterozygous *Crx mouse* ("+/-") will develop outer segments but they are shorter than the WT. Furthermore they have reduced cone and rod activity and that can be seen in ERG, which have low amplitudes compared with the wild type (Furukawa *et al*, 1999).
In the *Crx Knock-Out* mouse ("-/-") mutants, photoreceptor-specific genes, such as the ones that encode for: rhodopsin, cone opsins, rod transducin α-subunit, cone arrestin and recoverin, will be observable with the exception of the ones that encode for the green/red cone opsin, albeit their expression will be downregulated in these mice (Furukawa *et al*, 1999). However the upregulation happens for other genes, e.g. *Cnga3* and *Neurod* (Furukawa *et al*, 1999). Thus, CRX is both a transcription factor and a repressor protein. Repression happens by its binding to the operator's gene, preventing it from being expressed and its promoter to be recognized by RNA polymerase, thereby turning off the gene.

1.5. Purpose and Hypotheses

The aim of the human experiment was to judge the qualitative (smoothness, temperature, stiffness and sliperiness), affective (pleasantness) and mnestic features of touch when haptically exploring surfaces of different textures. Tactile memory was tested immediately after the presentation of the different textures for testing short-term memory. We hypothesized that congenitally blind subjects would report higher affect ratings and higher scores on the qualitative descriptors of the touched surfaces compared to sighted subjects, because it was shown that blind have a hyperacuity in a haptic and tactile discrimination tasks (Goldreich & Kanics, 2003 and Alary et al, 2009).
Furthermore we expected that blind subjects would outperform the sighted control subjects in short-term tactile memory, as reflected by a higher percentage of correctly recognized materials and would have faster response times when discriminating earlier presented from new materials, due to the their recruitment of the visual cortex during haptic texture discrimination.
The aim of the animal experiment was to compare the cellular density and other differences between the two genotypes: *Crx-/-* and the wt mice, namely in the primary somatosensory cortex (S1) and in the primary visual cortex (V1), through observations through microscopy analysis.

2. Materials and Methods

This chapter comprises the materials and methods used in the two experiments done during the development of this thesis.

2.1. Animal experiment

This preliminary study was done in the School of Optometry, in the University of Montreal.

2.1.1. Methodology

2.1.1.1. Animals

Brain mouse tissues were obtained from 3 mice, 2-3 months old, strain 129SV, which 1 was WT and 2 *Crx Knockout "(-/-)"*. The mice were kindly provided by Dr. Martin Rath, from the University of Copenhagen.

2.1.1.2. Tissue preparation

Mice were euthanized and cardiacally perfused. Their brains were preserved and frozen for immunohistochemistry.

Coronal sections were cut (20 µm) at -22ºC on a Leica CM3050S cryostat and mounted onto gelatinized subbed glass slides. The slides were then stored at -80ºC until used for the next step.

2.1.1.3. Staining (Cresyl)

2.1.1.3.1. Technique definition

Nissl staining is one of the most used stains worldwide in brain research. It requires the basic histological dye Cresyl violet.

The Nissl bodies or granules are located in the soma (nerve cell bodies) and dendrites and the Cresyl violet will bind to them and allow their visualization in bright-field microscopy. Besides this, it allows the analysis of cells, when there is a need to count them.

Thus, the soma and the dendrites will present a violet-purple colour, but not the glial cells.

After staining the slides can be conserved for many years. They can be afterwards analysed with a light microscope and photographed.

2.1.1.3.2. Protocol

6 slice-mounted fresh-frozen tissues, containing the somatosensory cortex, from both groups of mice were select to stain.

2.1.1.3.2.1. Materials

- Staining wells
- Slide holders
- Coverslips
- Prepared subbed slides with tissue
- Permount mounting media
- Reagents:
  1. Cresyl Violet Acetate (C1791-SG)
  2. Xylene
  3. 100% Alcohol
  4. 95% Alcohol
  5. 70% Alcohol
  6. ddH_2O

2.1.1.3.2.2. Procedure

Herein it is described the procedure:

1) The slides were put into holders, facing the same way, around 20 minutes before staining to allow them to warm to room temperature.
2) After that, the holders were put in wells containing the following solutions for the times indicated:
   i. Xylene (5 minutes)
   ii. 95% Alcohol (3 minutes)
   iii. 70% Alcohol (3 minutes)
   iv. Double distilled water (3 minutes)

v. Cresyl Violet Acetate (11 minutes)
vi. Double distilled water (3 minutes)
vii. 70% Alcohol (3 minutes)
viii. 95% (2 minutes)
ix. 100% Alcohol (1 dip)
x. Xylene (5 minutes)
xi. Slides cover-slip using Permount mounting media (Fisher Scientific; Pittsburgh, PA, USA).
xii. Slides were allowed to dry in the fume hood.

In this procedure, the Alcohol had the function of rehydrate on the first steps before the Cresyl Violate Acetate that will stain. The quick rinse with double distilled water removed the excess of the stain. The 70% and 95% Alcohol after the Cresyl Violet Acetate washed the slides and the 100% Alcohol dehydrated them. The last xylene was used to clear. The fume hood was used in all steps.

The last step was to examine the slides and to take pictures with a Leica DMRB under bright field illumination, of the somatosensory and visual cortex in both groups in order to compare them.

2.2. Human experiment

For this experiment its study and consent procedure was approved by the ethics committee for the city of Copenhagen, Denmark. Participants provided their written informed consent to participate in this study and were compensated for their time and effort.

The experimental design of the two human studies is a matched case-control study in which the congenitally blind subjects were matched to age –and sex-matched sighted controls.

2.2.1. Methodology

2.2.1.1. Location of the study

The study took place at the Brain Research and Integrative Neuroscience Laboratory (BRAINlab) located at the Panum Institute, Department of Neuroscience and Pharmacology, University of Copenhagen. The experiment took also place in the Dansk Blimdesamfund and in some blind participants' place.

2.2.1.2. Participants

Subjects were recruited from the BRAINlab's database of congenitally blind subjects or by advertisement. A total of 11 congenitally blind (3 females and 8 males (CB median age was 58) and 11 sex- and age- matched individuals with normal (or corrected to normal) vision and neurologically normal, with a median age of 49.

There were different origins of blindness among the congenitally blind participants: retinopathy of prematurity (n=1), glaucoma (n=3), LCA (n=2), premature birth (n=3) and RP (n=3).

None of the participants had any disease that could compromise their ability to feel the different textures and participate in the experiment.

All of the participants knew how to read Braille since a very young age (between 4-11), with the exception of four participants that learnt at the ages of 19, 20 and 38. All of the participants received some help in the daily life (cane, dog, at home ot at work), with the exception of one female participant that didn't receive any help and didn't have a dog. But, despite of this fact, all of them were very autonomous in their daily life, were educated above primary school and spoke very good english.

2.2.1.2.1 Inclusion and exclusion criteria

- Inclusion criteria:
    - More than 18 years of age.
    - Good medical and psychological health.
    - Sighted participants: Normal or corrected to normal vision.
    - Congenitally blind: Absence of sight at birth or within the first year of age with no residual light perception.
    - Late blind: Loss of vision after the age of 6, with no residual light perception.
- Exclusion criteria:
    - Presence or recent history of significant, actual or unstable medical, neurological and psychiatric disorders that may interfere with the central nervous system or peripheral nervous system (i.e. polyneuropathy or myotonic dystrophy).
    - For the blind participants: blindness of central origin (e.g. vascular or tumour lesions in the occipital cortex).

2.2.1.3. Stimuli

For this study 47 materials samples were created (see figures from 2.3 till 2.48) that vary in terms of their roughness, stickiness, smoothness among other sensations, that allow participants to perceive different sensations when touching them. Examples of materials that participants were asked to explore were wood, metal, fur, aluminium, paper, etc. All samples measure 3 x 3 cm and are mounted on a rigid backing (piece of cardboard). In doing so it will avoid that the subjects recognize from which object is the material from and they would not feel different shapes and would be focused only in the texture of the materials. Therefore haptic texture discrimination would be tested.

2.2.1.4. Procedure

Before the experiment could begin, the blind or blindfolded sighted control was seated in a comfortable chair in a quiet room with only the participant and the examiner present. Each participant was tested one at a time. In order for the participants to rate qualitative characteristics of a number of different materials, they had the different rating scales of the study explained and were familiarized with the qualitative characteristics by trying some examples of the extreme points, e.g. fur is 5 because it is a very pleasant material to touch.

In this study it was also explored the somatosensory abilities of the congenitally blind subjects, using mechanosensitive receptors that contribute to discriminative touch, namely the Merkel neurite complex, which signals are carried by SAI afferents (Connor & Johnson, 1992 and Yoshioka et al, 2001 in Alary *et al*, 2009) and also, but contribuiting less, so with a small role, the Meissner and Pacinian corpuscles, which mechanoreceptive afferents are RA and PC (Yoshioka *et al*, 2001 in Alary *et al*, 2009).

The subjects and controls of this study had to quantitatively score the different materials of the qualitative characteristics of the material, i.e. pleasantness, smoothness, temperature, slipperiness and stiffness. After exploring the materials with their second and third digits the subjects and controls used a quantifiable 11-level scale from -5 till 5, where -5 and 5 were considered the very extremes of responses and 0 being neutral. Subjects were not asked to name the materials they explored.

Table 2.1. Meaning of the rating scale used to classify quantitatively the different factors.

Factor	-5	0	5
Pleasantness	Unpleasant	Neutral	Very pleasant
Smoothness	Rough	Neutral	Very smooth
Temperature	Cold	Neutral	Warm
Sliperiness	Unslippery	Neutral	Very slippery
Stifness	Soft/Maleable/Compressive	Neutral	Very stiff

To begin with the subjects and controls were presented with a few test materials that were not part of the experimental set and that simply were used to assure that participants had correct understanding of the task instructions and the usage of the rating scales (see tables 2.1 and 2.2) . In this period of time participants also learnt the duration that they would have to explore each material.

Table 2.2. Objects used as examples for better comprehension of the rating scale by the subjects.
(The numbers after the materials can be checked in the appendix).

Factors	-5	5
Pleasantness	Sandpaper P1000 (23)	Fur (19)
Smootheness	Exfoliating sponge (5)	Leather (43)
Temperature	Marble (47)	Wool (1)
Sliperiness	Sandapaper P1000 (23)	Wood (45)
Stifness	Kitchen sponge (12)	Metal (40)

After the initial information, consent and instructions, testing could begin. A total of 20 different materials were presented one at the time. The order of presentation of the different materials across the subjects was in a random order, but the same for each participant. The participant were asked to touch in a total of 15 seconds in each material, divided 5 times so they could each time rate for each factor, they touched the materials with the dominant hand and with the fingertips of digit 2 and 3. After touching the material the participant rated it and touched the next one, doing the same procedure for the 20 materials. The manner in which, the velocity at which and the force of stimulus contact the materials were not controlled. Hence they were allowed to explore the materials under the conditions set out above, however in their own manner. Participants weren't advised to remember the textures that they touched and explored and were not told the name of the objects that each material belonged to in order to avoid mental attachement regarding their tactile memory and their intentional learning (Röder, 2001) considering that after 10 minutes of

presentation of the test materials, short-term tactile memory would be tested. A total of 10 stimuli of the initial 20 were mixed with 10 new stimuli that participants had not been exposed to and their order was selected beforehand. Using a forced choice paradigm, participants had to indicate whether they had felt the stimuli before or not by just answering an assertive “yes” or “no” to the question “Did you touch this material during your earlier session?”. To avoid the obvious risk of random selection, we asked the participants to indicate the certainty of their response by quantifying their choice in a 11-point rating scale in which “0” means a complete guess in contrast with “10” that means very sure of the answer, therefore they were very confident if it was the same or a new material that they touched in the second session. For the memory task participants had a maximum of 10 seconds that they could use to explore each material and they weren’t given any feedback regarding their answers.

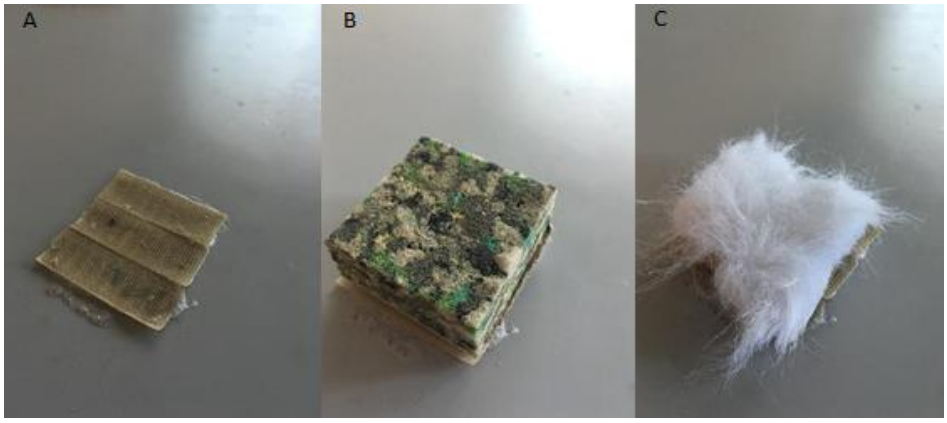

Fig. 2.1. (A) piece of velcro that was glued on the table in order to fix the materials on it. The materials had also in their base another piece. (B) and (C) are materials fixed on the table ready to be touched by the volunteers.

Fig.2.2. Blindfolded volunteer participating in the experiment.

Fig. 2.3. Texture 1.

Fig. 2.4. Texture 2.

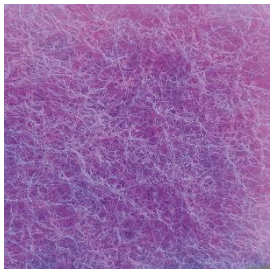

Fig. 2.5. Texture 3.

Fig. 2.6. Texture 4.

Fig. 2.7. Texture 5.

Fig. 2.8. Texture 6.

Fig. 2.9. Texture 7.

Fig. 2.10. Texture 8.

Fig. 2.11. Texture 9.

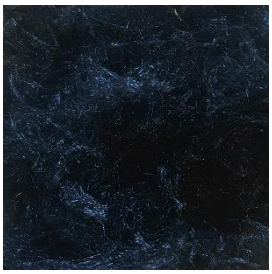

Fig. 2.12. Texture 10.

Fig. 2.13. Texture 11.

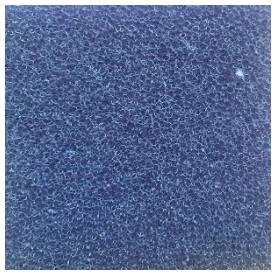

Fig. 2.14. Texture 12.

Fig. 2.15. Texture 13.

Fig. 2.16. Texture 14.

Fig. 2.17. Texture 15.

Fig. 2.18. Texture 16.

Fig. 2.19. Texture 17.

Fig. 2.20. Texture 18.

Fig. 2.21. Texture 19.

Fig. 2.22. Texture 20.

Fig. 2.23. Texture 21.

Fig. 2.24. Texture 22.

Fig. 2.25. Texture 23.

Fig. 2.26. Texture 24.

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Fig. 2.30. Texture 28.

Fig. 2.31. Texture 29.

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Fig. 2.39. Texture 38.

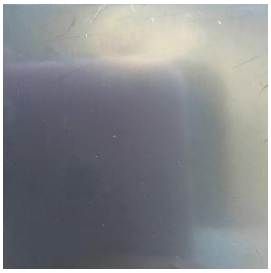

Fig. 2.40. Texture 39.

Fig. 2.41. Texture 40.

Fig. 2.42. Texture 41.

Fig. 2.43. Texture 42.

Fig. 2.44. Texture 43.

Fig. 2.45. Texture 44.

Fig. 2.46. Texture 45.

Fig. 2.47. Texture 46.

Fig. 2.48. Texture 47.

2.2.1.5. Statistics

Regarding the statistics used in order to interpret the results, first we used descriptive statistics (median and IQR) in order to study the characteristics of both groups. Afterwards Shapiro-Wilk test was applied to the population in order to study its distribution and since there were less than 2000 subjects, the population was not normally distributed so non parametric tests were used in the next step. These were Mann-Whitney U test and Fisher's Exact test. Thus the significance of the short-term memory test and the different ratings for the materials were verified. The first one was also verified with the signal detection theory.

3. Results

3.1. Results from the animal experiment

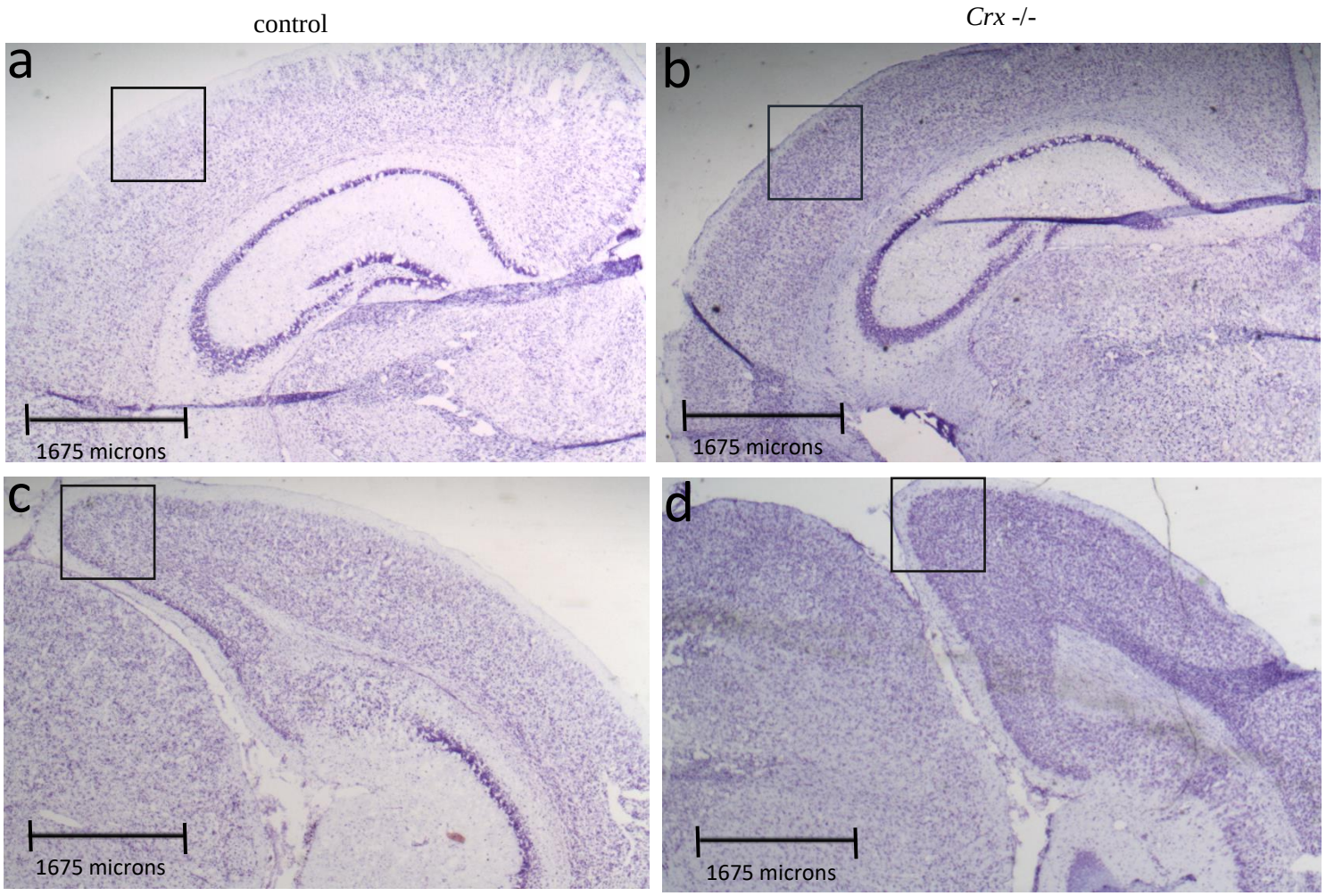

Fig.3.1.Coronal sections of the primary somatosensory cortex (S1) and visual cortex (V1) with Nissl-substance staining in adult wt (a) and (c) and in the *Crx Knock-Out* mice ("-/-") (b) and (d) (2.5x Magnification).The squares represent where there was an enlargement that originate pictures (e), (f), (g) and (h), respectively.

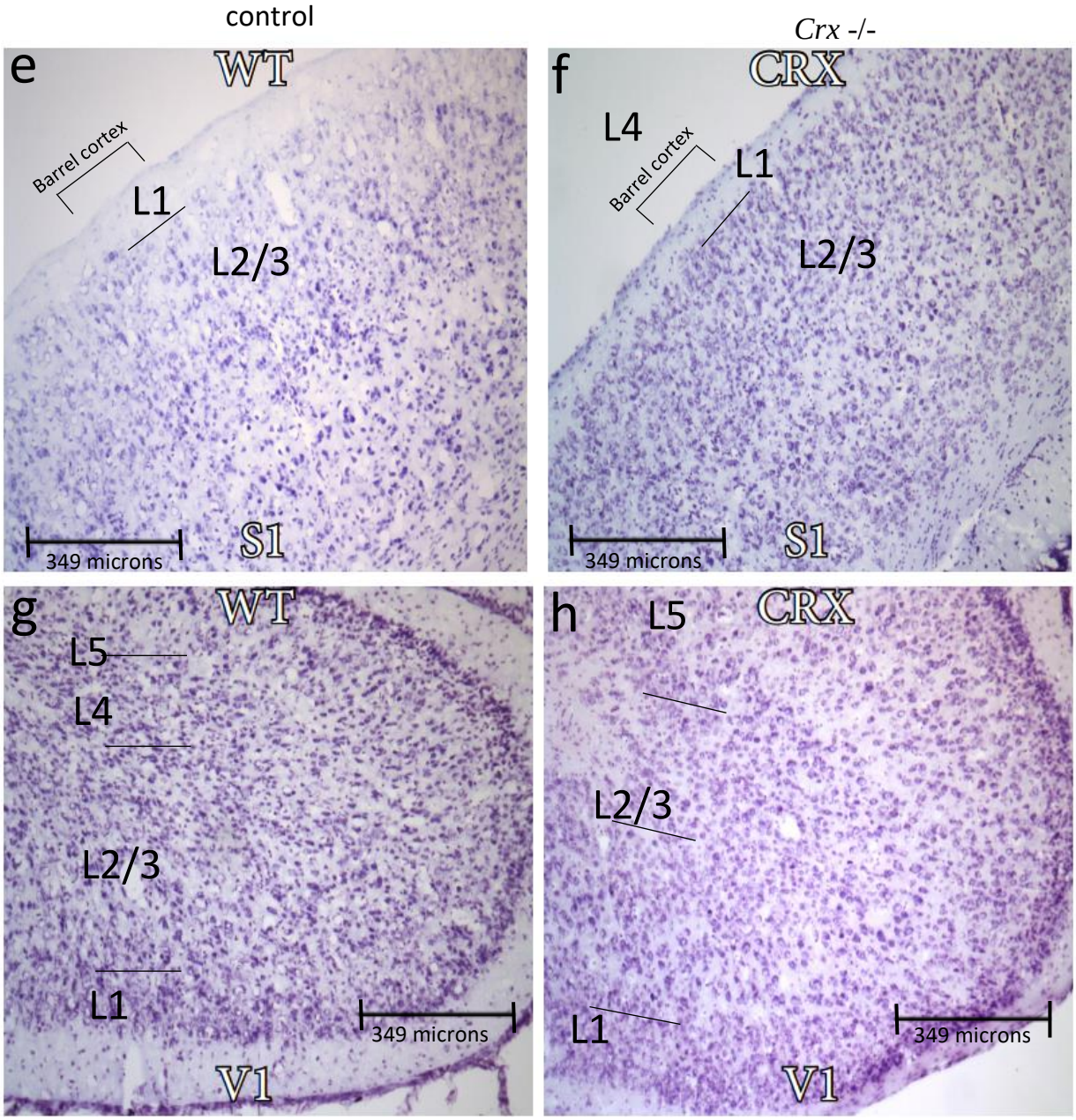

Fig. 3.2. Coronal sections of the primary somatosensory cortex (S1) and visual cortex (V1) with Nissl-substance staining in adult wt (e) and (g) and in the *Crx Knock-Out* mice ("-/-") (f) and (h) (10x Magnification).

The Nissl staining in figures 3.1 and 3.2 show differences in cellular density in the cortical layers of S1, namely in layers L1 and L2/3 in *Crx-/-* and wt mice. The wt mice had thicker L1 than the *Crx-/-* mice as seen in figure 3.2e and f. Another nuclei count of L2/3 show more cells in the *Crx-/-* mice than in the wt. Despite this fact both groups of cells have a normal appearance. To have a more reliable nuclei count optic density is needed. This was unfortunately not available at

BRAINlab. L1 and L2/3 in V1 are very homogenous in microscopic appearance in both genotypes of mice as can be seen in the figures above.

These preliminary results suggest that there aren't morphological changes nevertheless there were some changes observed in a non-visual region of the cortex in the two groups. These findings confirm that there is an ongoing activation of the visual cortex nuclei in the visually deprived mouse brain, meaning that no degeneration took place in this area even without visual stimulation. This early representation of the S1 and V1 in the *Crx-/-* and wt mice is in accordance with the recruitment of deprived areas in blind humans, as seen in *crossmodal plasticity*, described in the chapters above. As expected, the deletion of the *Crx* gene did not have any effect on the *cytoarchitecture* or early nuclei count of the primary visual cortex (V1) in the adult mouse.

3.2. Results from the human experiment

Table 3.1. General characteristics of the study population.

	CB	SC
Age, median (IQR), years	58 (29)	49 (18)
Gender, n • F • M	3 8	3 8
Blind and sighted subjects, n	11	11

The study population as it can be seen in table 3.1 and calculated by Shapiro-Wilk test it is not normally distributed so we cannot use the student´s t-test, but have to analyse our data with a nonparametric test, namely the Mann-Whitney U test.

Surprisingly our results in the Mann-Whitney U test show that contrary to hypothesized, there were no significant differences in the rating of any of the different factors between sighted and congenitally blind subjects (pleasantness (Sig = 0,562), smoothness (Sig = 0,151), temperature (Sig = 0,365), slipperiness (Sig = 0,949) and stiffness (Sig = 0,365)). When analysing each material by itself, the blind rated the factor slipperiness for the material 44 (Sig = 0,040), the factor smoothness for the material 46 (Sig = 0,028) and the factor pleasantness the materials 25

(Sig = 0,034) and 41 (Sig = 0,019) significantly more extreme. The kind of material referred can be checked in the procedure chapter and in the appendix.

Furthermore, there were no scores significantly different between congenitally blind and sighted subjects in the short-term memory task (Mann-Whitney U test; Sig. = 0,217).

There weren't significant differences in the certainty of the answers between these 2 groups (Mann-Whitney U test; Sig. = 0,748).

As it can be seen in figure 3.3 the mean score of certainty of the answers in the short-memory task was 6 for congenitally blind and 7 for sighted controls.

The short-term memory task was also analysed using signal detection theory and the analysed outcomes were hit rate, false identification and d'. The hit rate is the number of correctly remembered old materials, while the false identification is the number of falsely remembered distracting materials (table 3.2.) The hit rate and false identification rate were used to calculate the sensitivity index (d'). d' is calculated through this formula: [d'=z(H) – z(F)]' (Stanislaw and Todorov, 1999). d' is dimension less, but a high score indicates a higher ability to separate old materials from new materials. There were no significant differences between the hit rate, false identification and d' (figures 3.17, 3.18, 3.19 and appendix).

All analyses were carried out in IBM SPSS Statistics 24 and the statistical significant level was set out $p \leq 0.05$.

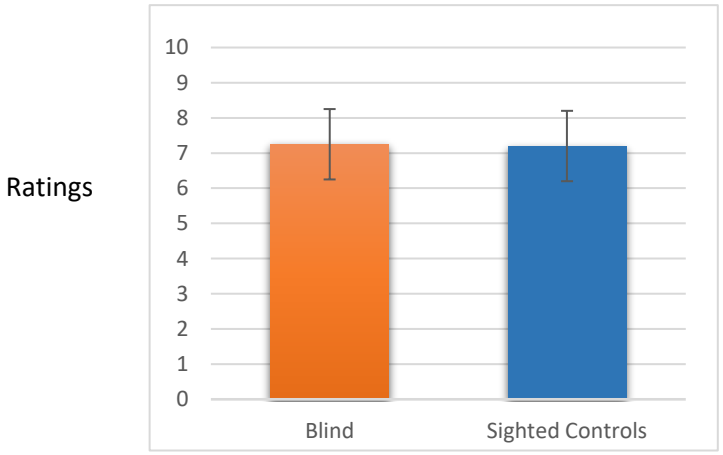

Fig. 3.3. Median (IQR) score of certainty of the answers.

In figure 3.4 it can be seen the median score of the short-memory task, that was 13 for sighted controls and 14 for blind. The score for each individual was calculated by the sum of 1 point for each material correctly identified (new or old). There were 20 materials. The median of total old materials and new materials correctly identified can be seen below in figures 3.5 and 3.6, respectively. In figure 3.5 the median of total old materials correctly identified by sighted controls was 7 and for blind as well. For the new materials the median was the following: sighted controls 6 and blind as well.

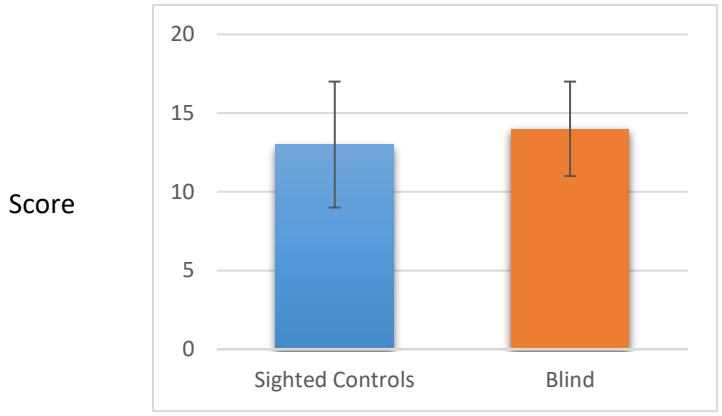

Fig. 3.4. Median score (IQR) of the memory task. The bars represent the IQR.

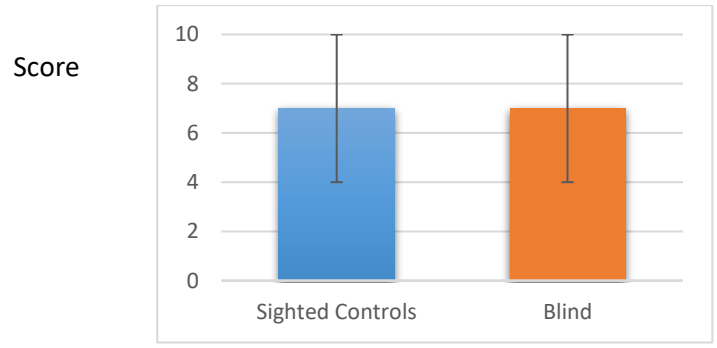

Fig. 3.5. Median (IQR) of total old materials correctly identified. The bars represent the IQR.

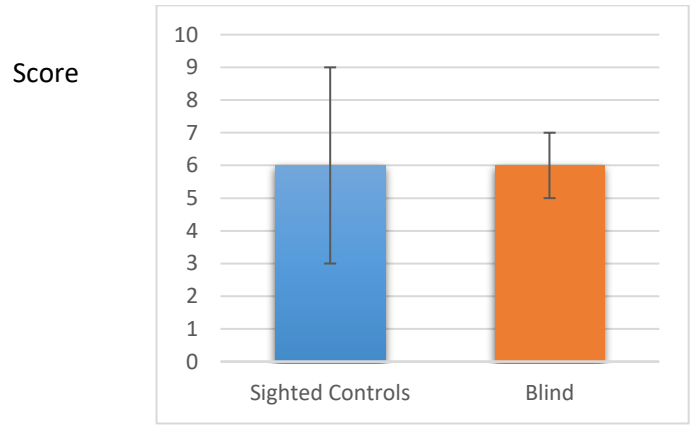

Fig. 3.6. Median (IQR) of total new materials correctly identified. The bars represent the IQR.

The total mean for each factor for each group can be seen in figures 3.7, 3.9, 3.11, 3.13 and 3.15. For the factor pleasantness median for sighted controls and blind were: 48 and 49, respectively. For smoothness, temperature, slipperiness and stiffness were: 66 and 61, 41 and 33, 64 and 60, 66 and 60.

The mean rate for the different factors for each material in both groups can be seen in figures 3.8, 3.10, 3.12, 3.14 and 3.16.

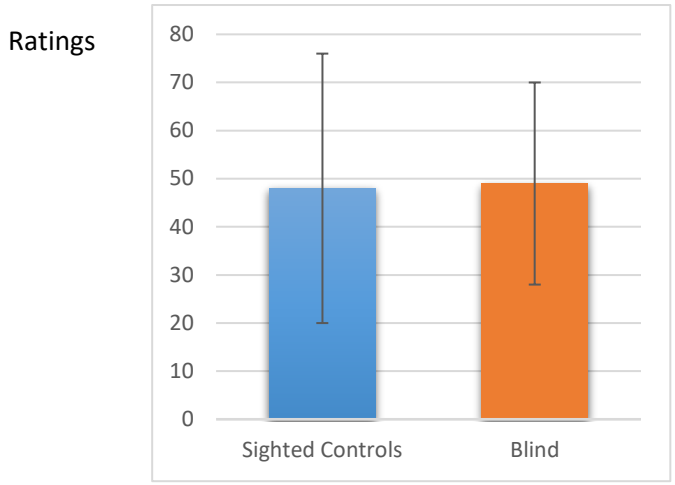

Fig.3.7. Median of the absolute rating of pleasantness. The bars represent the IQR.

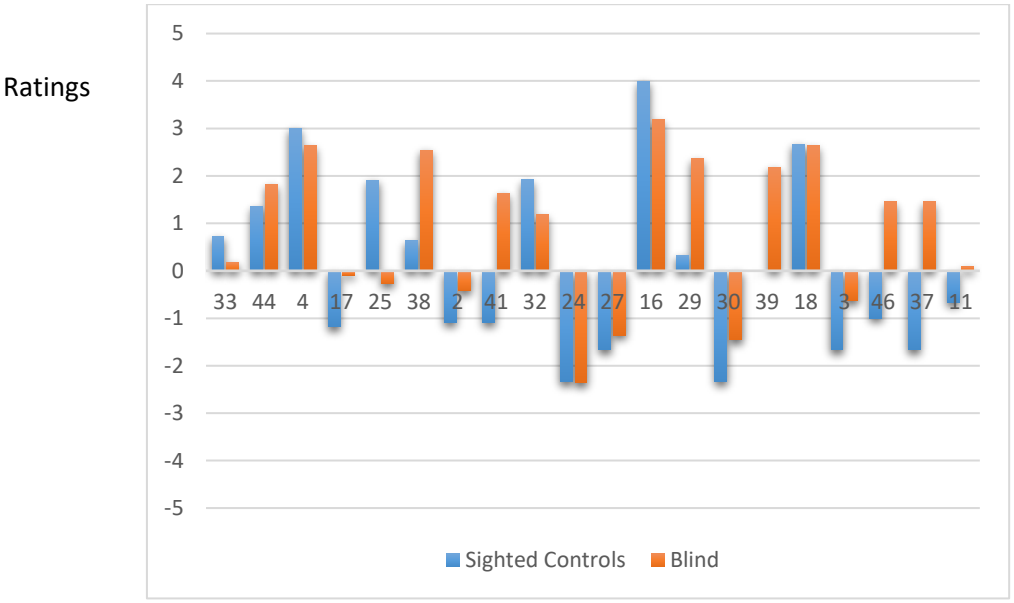

Fig.3.8. Mean rates for the factor pleasantness for each material analysed by Fisher's Exact Test.

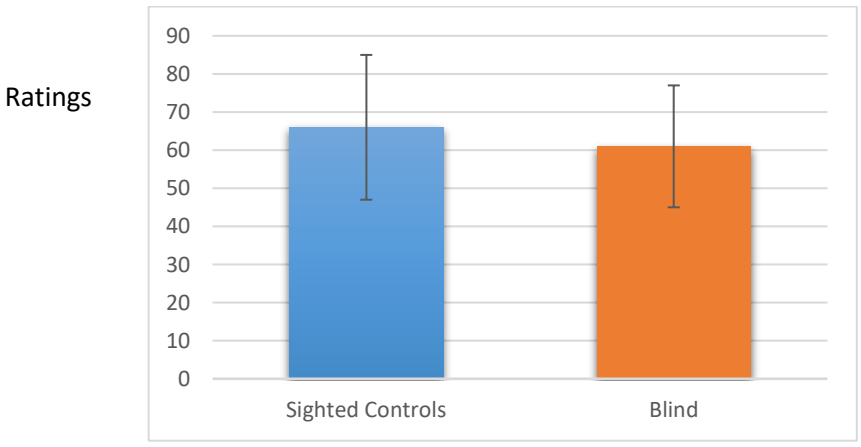

Fig.3.9. Median (IQR) of the absolute rating of smoothness. The bars represent the IQR.

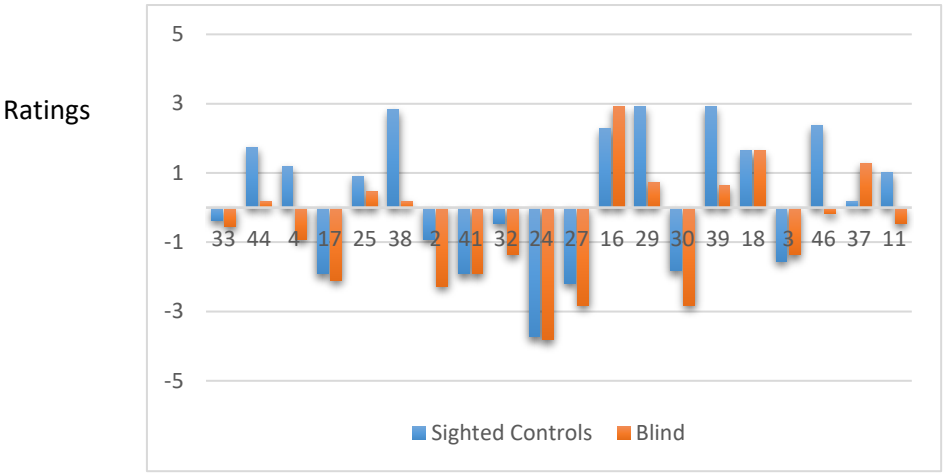

Fig.3.10. Mean rates for the factor smoothness for each material analysed by Fisher's Exact Test.

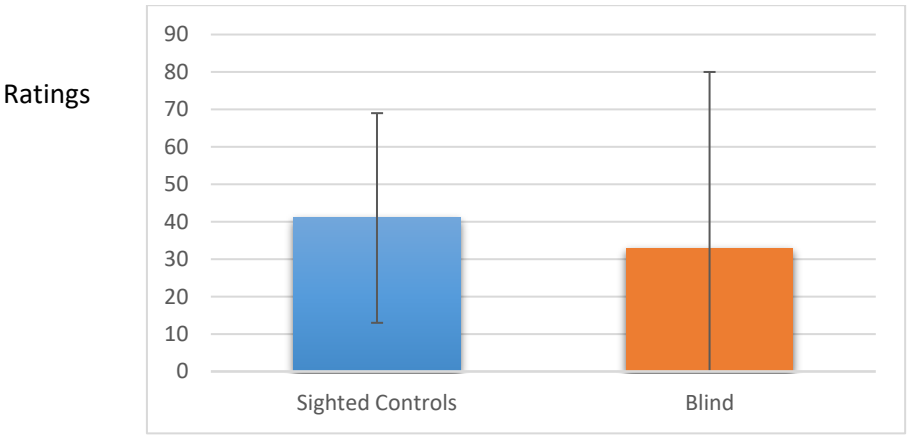

Fig.3.11. Median (IQR) of the absolute rating of temperature. The bars represent the IQR.

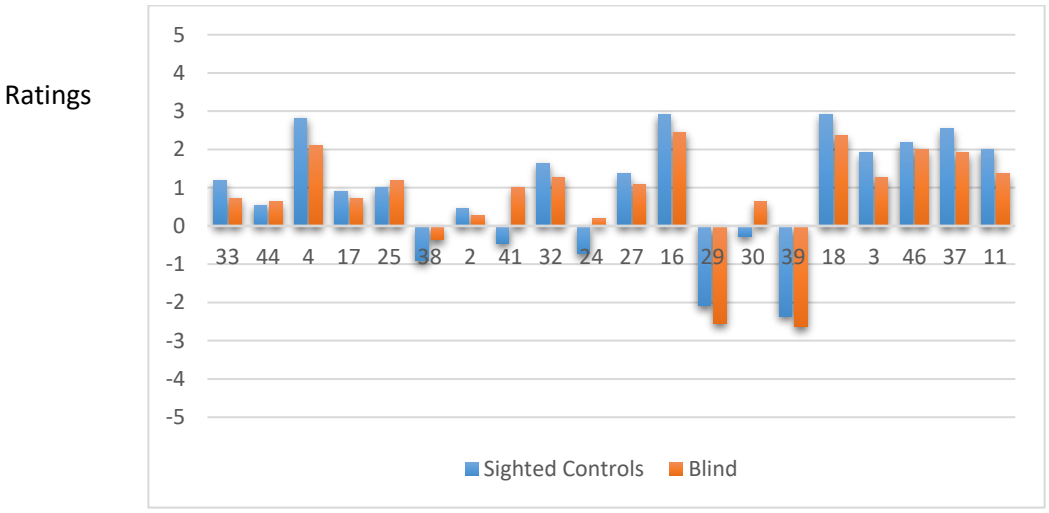

Fig.3.12. Mean rates for the factor temperature for each material analysed by Fisher's Exact Test.

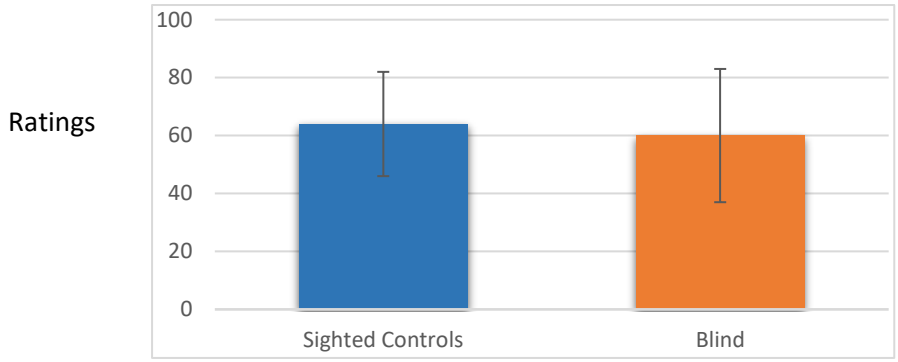

Fig.3.13. Median (IQR) of the absolute rating of slipperiness. The bars represent the IQR.

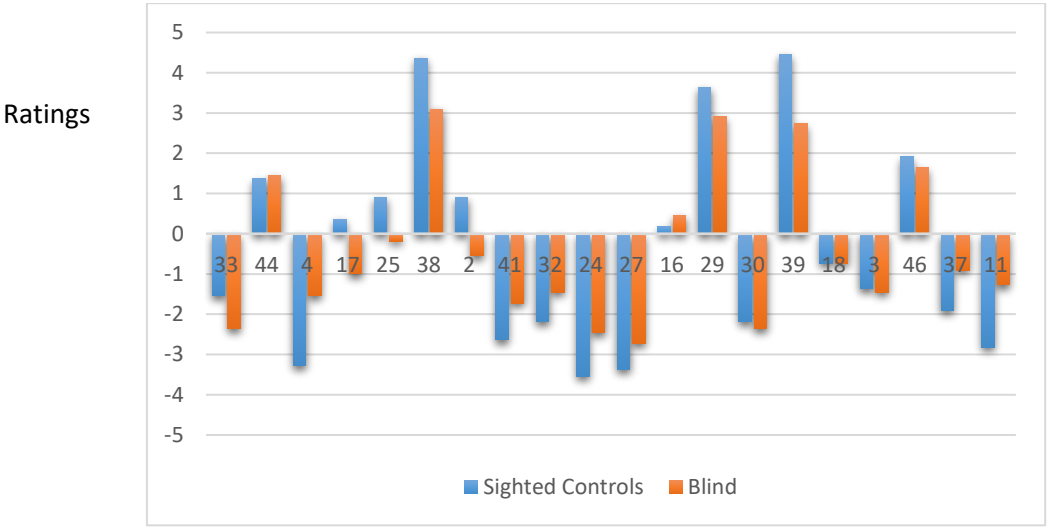

Fig.3.14. Mean rates for the factor slipperiness for each material analysed by Fisher's Exact Test.

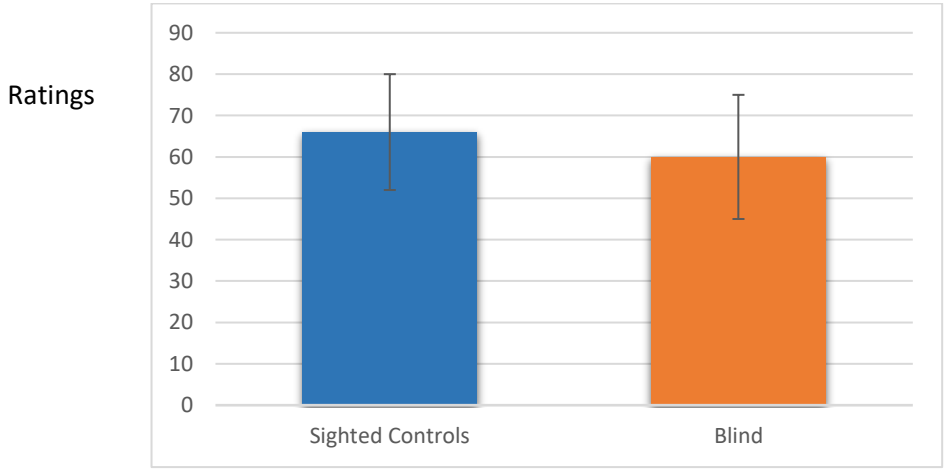

Fig.3.15. Median (IQR) of the absolute rating of stiffness. The bars represent the IQR..

Fig.3.16. Mean rates for the factor stiffness for each material analysed by Fisher's Exact Test.

Table 3.2. Overview of the possible outcome in the memory test:

	Target material	*Distracting material*
Positive response	Hit	False Identification
Negative response	Missed target material	Correctly rejected

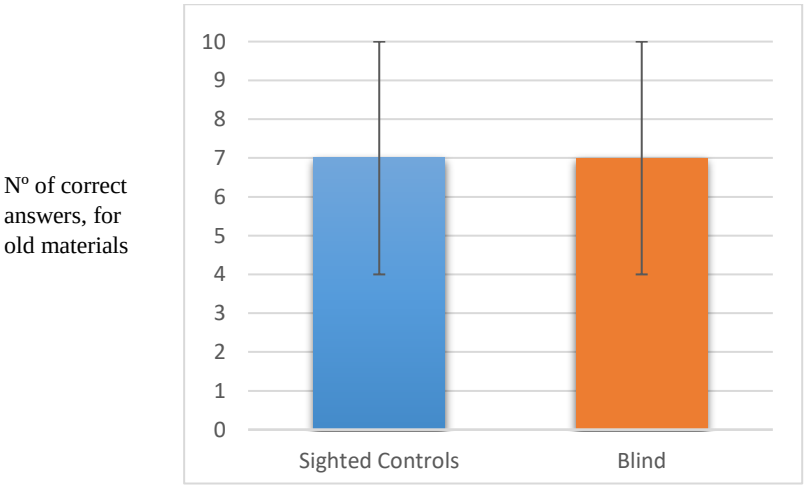

Fig.3.17. Median (IQR) hit rate after 10 minutes. The bars represent the IQR.

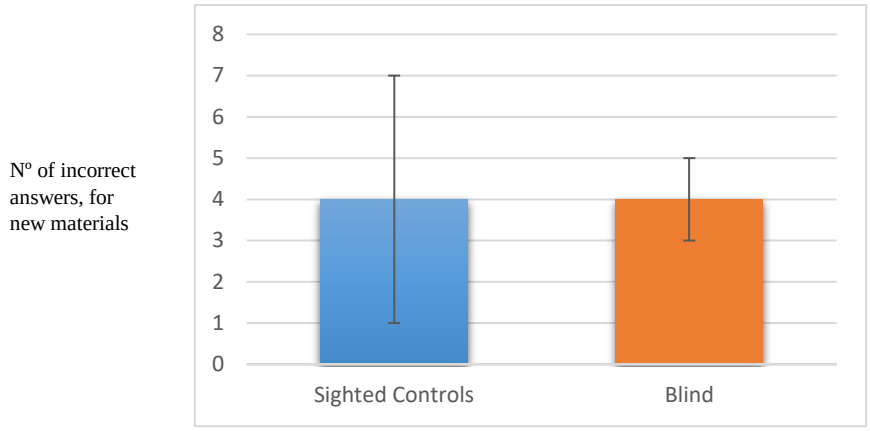

Fig.3.18. Median (IQR) false identification after 10 minutes. The bars represent the IQR.

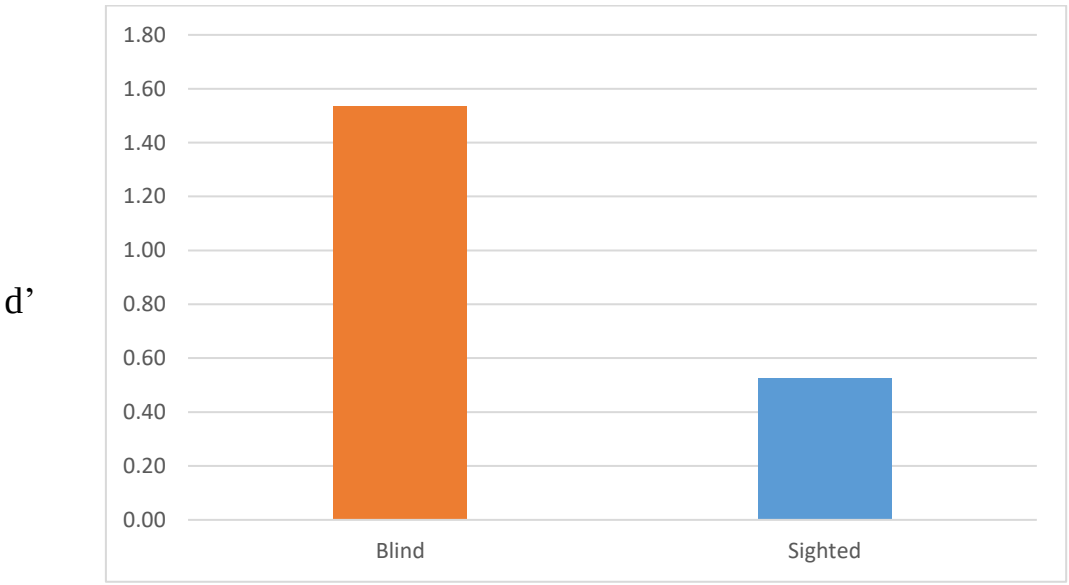

Fig.3.19. Median (IQR) d’ after 10 minutes for blind and sighted subjects.

4. Discussion

This study compare short-term tactile memory task in congenitally blind individuals with age- and sex matched sighted controls. Results show that congenitally blind didn't outperform their counterparts in the short-term memory task, which indicates that there is no difference in the short-term tactile memory and that it is equally as important in blind and in sighted individuals.

Contrary to our hypothesis, congenitally blind didn't rate more extremely than sighted controls in the different factors for each material neither showed more certainty in their answers.

These very surprising results indicate that the superior ability of congenitally blind individuals in a variety of non-visual tasks doesn't apply to this case: short-term tactile memory task and haptic discrimination.

When reviewing literature in the field of study, as earlier stated, it seems that multiple sensory modalities are enhanced by a long-term deprivation of vision. Tactile acuity was no exception. Goldreich and Kanics (2003) tested 90 participants and demonstrated that blind subjects are better than sighted in discriminating the orientation of a grating applied to the fingertip. Voss (2016) showed that the occipital cortex was primarily right lateralized, regardless of the stimulated hand, supporting previous evidence for a right-sided hemispheric specialization of the occipital cortex of blind individuals for the processing of tactile and haptic inputs. Subjects performed tactile angle size discriminations. This study was conducted with 15 EB and 14 healthy control. Norman & Bartholomew (2011) studied the tactile grating orientation discrimination, in order to determine tactile acuity and also haptic three-dimensional (3-D) shape discrimination in the blind (congenital, early, and late). In their study they used 32 participants. Their results show that the improvements in haptic 3-D shape discrimination only occurred for the early-onset and late onset blindness groups but congenital blind doesn't outperform the sighted. These 3 studies exploring tactile abilities in the blind were the ones most resembling in current literature.

The study population in this thesis was a relatively low (11 subjects in each group) age and sex-matched sample. All the blind subjects suffered from visual deprivation from birth or till 2 years old. The population sample wasn't normally distributed so nonparametric statistics were used in order to analyse our results. A bigger study sample is needed in the future to extrapolate results into a relevant cohort.

Our study will contribute to increase knowledge in this field, as there are no studies related to short-term tactile memory and haptic discrimination in congenitally blind subjects and there is a lack of prior research studies on this topic.

Our results were surprising, deepening our understanding of sensory compensation in congenitally blind subjects as neuronal changes in the visually deprived brain. This was done

using molecular genetics in an animal experiment and human subjects to further analyse our results in a *crossmodal plasticity* paradigm. Furthermore current literature was reviewed in order to understand and reflect on the implications of these results on future research and rehabilitation of blind.

To confirm our results it could be interesting to include additional studies with fMRI or rTMS that could reveal if the visual cortex is or isn't activated in haptic and tactile memory tasks like this one. Moreover a second memory task with 1 week delay could be included in a future study, testing long-term tactile memory and exploring how tactile memory develops in visually deprived brains. With more blind subjects, a comparison of young and older blind as well as gender differences could be revealed, besides more blind subjects would give clearer insight in the visually deprived brain and making it easier to extrapolate to all blind.

There has now been performed series of studies exploring *crossmodal plasticity* in congenitally blind subjects. Most of this conclude that part of the blinds outperforming of the sighted in non-visual tasks is due to recruitment of the visual cortex. It could be interesting to map the changes of *crossmodal plasticity* of the cortical areas in the reshaped visually deprived brain. This could potentially be done through stimulating blind subjects with different sensory modalities while simultaneously doing fMRI and rTMS.

Finally further research is needed to better characterize the dependence of *crossmodal plasticity* on the time course of blindness and to determine the perceptual consequences of *crossmodal* activation.

Potential bias of this study needs to be taken into account.
In our experiment bias in concepts could have made our rating more similar between all subjects, as it is known that people have a special preference for digits 0 and 5, so ratings could be chosen more frequently with these numbers. Conversion from a qualitative factor to a quantitative scale can be a bias where each individual vary, despite being explained and familiarized with the procedure before testing began.
The Hawthorne effect may have had a bigger influence on this study than other types of study, because blindfolding sighted controls may change their haptic perception.
In order to avoid bias in the experiment a blindfold was asked to the participants with sight to use. We chose quiet surroundings in order to eliminate outside stimuli in the experiment. Also sex and age were matching. The participants were familiarized with the experiment.
In conclusion, our results show no significant differences in haptic discrimination and short-memory tactile task but due to study limitations and limited literature in the field more research is needed with more subjects and more sophisticated technology.. Furthermore our animal study had initial results showing no structural differences in L2/L3 in the visual deprived brain.

"For me, as a blind person, I find it easier to learn through tactile tools so I would like that to happen in the future. I learn faster with tactile devices and right now we are using mostly our hearing in order to learn."

Annie, congenitally blind, 42 years old

5. References

Abraira, V. and Ginty, D. 2013. The Sensory Neurons of Touch. Neuron. 79 (4): 618-39.

Alary, F. *et al.* 2009. Tactile acuity in the blind: A closer look reveals superiority over the sighted in some but not all cutaneous tasks. Neuropsychologia. 47: 2037-2043.

Bolanowski, S. *et al*. 1988. Four channels mediate the mechanical aspects of touch. J Acoust Soc Am. 84(5): 1680–1694.

Buonomano, D. *et al.* 1998. Cortical plasticity: from synapses to maps. Annu Rev Neurosci. 21: 149-86.

Chau, K.Y. *et al*. 2000. Functional domains of the cone–rod homeobox (CRX) transcription factor. J. Biol. Chem. 275: 37264–37270.

Chen, S. *et al*. 1997. Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. Neuron. 19(5):1017–1030.

Chen, S. *et al.* 2002. Functional analysis of cone-rod homeobox (CRX) mutations associated with retinal dystrophy. Hum Mol Gen. 11: 873-884.

Connor, C.E. and Johnson, K.O. 1992. Neural Coding of Tactile Texture: Comparison of Spatial and Temporal Mechanisms for Roughness Perception. The Journal of Neuroscience. 12(9): 3414-3426.

De Volder, A.G. *et al*. 1999. Changes in occipital cortex activity in early blind humans using a sensory substitution device. Brain Research. 826(1): 128-34.

Furukawa, T. *et al.*1997. Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. Cell. 91: 531-541.

Furukawa, T. *et al*. 1999. Retinopathy and attenuated circadian entrainment in crx-deficient mice. Nature Genetics. 23:466–470.

Gardner, E. 2010. Touch. Encyclopedia of Life Sciences (ELS). John Wiley & Sons. 1-12.

Gescheider, G. 2002. A four-channel analysis of the tactile sensitivity of the fingertip: frequency selectivity, spatial summation, and temporal summation. Somatosensory & Motor Research. 19(2): 112-14.

Gogate, P. *et al.* 2011. Severe Visual Impairment and Blindness in Infants: Causes and Opportunities for Control. Middle East Afr J Ophthalmol. 18(2): 109–114.

Goldreich, D. *et al.* 2003. Tactile Acuity in Enhanced in Blindness. The Journal of Neuroscience. 23(8):3439 –3445.

Hubel, D.H. and Wiesel, T.N. 1964. Effects of Monocular Deprivation in Kittens. Naunyn-Sehmiedebergs Arch. exp. Path. u. Pharmak. 248: 492-497.

Iversen, K. *et al*. 2015. Enhanced Chemosensory Detection of Negative Emotions in Congenital Blindness. Neural Plasticity Volume 2015. 2015:469750.

Jamali, N. *et al.* 2010. Material Classification by Tactile Sensing using Surface Textures. EEE Transactions on Robotics. 27(3): 508-521.

Kandel, E. *et al*. 2000. Principles of neural science, 4th ed. McGraw-Hill, New York, United States of America.

Kupers, R. *et al*. 2011a. The nature of consciousness in the visually deprived brain. Front Psychol. 2(19).

Kupers, R. *et al*. 2011b. Neural correlates of olfactory processing in congenital blindness. Neuropsychologia. 49(7): 2037-44.

Kupers, R. *et al.* 2014. Compensatory plasticity and cross-modal reorganization following early visual deprivation. Neuroscience and Biobehavioral Reviews. 41: 36–52.

Obrist, M. *et al*. 2013. Talking about Tactile Experiences. ACM New York. 1659-1668.

Norman, JF. *et al.* 2011. Blindness enhances tactile acuity and haptic 3-D shape discrimination. Atten Percept Psychophys. 73:2323–2331.

Pascual-Leone, A and Torres, F. 1993. Plasticity of the sensorimotor cortex representation of the reading finger in Braille readers. Brain. 116: 39-52.

Pearson, W.R. 2013. An introduction to sequence similarity ("homology") searching. Current protocols in bioinformatics. Editoral board, Andreas D. Baxevanis et al. Chapter 3: Unit3.1.

Pons, T. 1996. Novel sensations in the congenitally blind. Nature. 380(6574): 479 – 480.

Ptito, M. *et al*. 2012. Sensory Deprivation and Brain Plasticity. Neural Plasticity. 2012:810370.

Rauschecker, J. 1995. Compensatory plasticity and sensory substitution in the cerebral cortex. Trends Neurosci. 18: 36-43.

Raz, N. *et al*. 2007. Superior Serial Memory in the Blind: A Case of Cognitive Compensatory Adjustment. Current Biology. 17: 1129–1133.

Röder, B. et al, 1997. Different cortical activation patterns in blind and sighted humans during encoding and transformation of haptic images. Psychophysiology. 34(3): 292-307.

Sadato, N. *et al*. 1996. Activation of the primary visual cortex by Braille reading in blind subjects. Nature. 380(6574): 526-8.

Sadato, N. *et al*. 1998. Neural networks for Braille reading by the blind. Brain. 121 (Pt 7): 1213–1229.

Slimani *et al*, 2015. Enhanced heat discrimination in congenital blindness. Behavioural Brain Research. 283: 233-237.

Snyder, P.J. and Whitaker, H.A. 2013. Neurologic heuristics and artistic whimsy: the cerebral cartography of Wilder Penfield. J Hist Neurosci. 22(3): 277-91.

Sjølie, A. *et al.* 2013. Praktisk oftalmologi 3. udgave. Gads Forlag. København K.

Stanislaw, H. and Todorov, N. 1999. Calculation of signal detection theory measures. Behavior Research Methods, Instruments, & Computers. 31(1): 137-149.

Théoret, H. *et al*. 2004. Behavioral and neuroplastic changes in the blind: evidence for functionally relevant cross-modal interactions. Journal of Physiology. 98(1-3): 221–233.

Tran, Ne. *et al.* 2014. Mechanisms of blindness: animal models provide insight into distinct CRX-associated retinopathies. Dev Dyn. 243:1153–1166.

Uhl, F. *et al*. 1991. On the functionality of the visually deprived occipital cortex in early blind persons. Neuroscience Letters. 124(2): 256-259.

Uhl, F. et al, 1993. Increased regional cerebral blood flow in inferior occipital cortex and cerebellum of early blind humans. Neuroscience Letters. 150(2): 162-4.

Voss, P. 2013. Sensitive and critical periods in visual sensory deprivation. Front Psychol. 4:664.

Voss, P. 2016. Crossmodal processing of haptic inputs in sighted and blind individuals. Front. Syst. Neurosci. 10:62.

Wanet-Defalque M.-C., *et al*. 1988. High metabolic activity in the visual cortex of early blind human subjects. Brain Res. 446(2):369–373.

6. Appendices

Appendix 1:

Table 6.1. Materials and their identification number:

Material	Number	1st Session	2nd Session	3rd session	Used as example
Wool	1				x
Aluminium paper	2	x	x	x	
Sponge	3	x			
Sew line	4	X			
Exfoliating sponge	5				X
Silk	6		X	x	
Exfoliating sponge 2	7		X	x	
Sandpaper P1000	8		X	x	
Tissue	9				
Fake fur	10		X	x	
Wool 2	11	x	X	x	
Kitchen sponge	12				x
Foam	13		X	x	
Kitchen paper	14		X	x	
Foam 2	15				
Foam 3	16	X	X	x	
Cup plastic	17	X	X	X	
Cotton	18	X	X	X	
Fake fur 2	19				X
Wool 3	20				
Wool 4	21		X	X	
Transparent plastic paper	22				

Sandpaper P100	23				X
Sandpaper P90	24	X	X	X	
Bubbles paper	25	X			
Foam 4	26		X	X	
Lace	27	X			
Cardboard	28				
Copper	29	X			
Sandpaper P50	30	x			
Wood 1	31				
Sew line 2	32	X			
Sponge 2	33	X	X	x	
Wood 2	34		X	X	
Foam 5	35				
Foam 6	36		X	X	
Foam 7	37	X	X	X	
Glass	38	X			
Metal	39	X			
Aluminium	40				X
Wood 3	41	X	X	X	
Wood 4	42				
Leather	43				X
Wood 5	44	X			
Wood 6	45				X
Styrofoam	46	X	X	X	
Marble	47				x

Appendix 2:

Table 6.2. Mann-Whitney U Test of the memory task – showing no significant difference in score comparing blind and sighted.

Ranks				
	Blind	N	Mean Rank	Sum of Ranks
Memory_score	Sighted	11	9,73	107,00
	Blind	11	13,27	146,00
	Total	22		

Test Statistics[a]	
	Memory_score
Mann-Whitney U	41,000
Exact Sig. [2*(1-tailed Sig.)]	,217[b]
a. Grouping Variable: Blind	
b. Not corrected for ties.	

Table 6.3. Mann-Whitney U Test of the rating task for the factor pleasantness – showing no difference comparing blind and sighted.

Ranks				
	Blind	N	Mean Rank	Sum of Ranks
Pleasantness	Sighted	11	10,68	117,50
	Blind	11	12,32	135,50
	Total	22		

Test Statistics[a]	
	Pleasantness
Mann-Whitney U	51,500
Exact Sig. [2*(1-tailed Sig.)]	,562[b]
a. Grouping Variable: Blind	
b. Not corrected for ties.	

Table 6.4. Mann-Whitney U Test of the rating task for the factor smoothness – shows no difference comparing blind and sighted.

Ranks				
	Blind	N	Mean Rank	Sum of Ranks
Smoothness	Sighted	11	13,50	148,50
	Blind	11	9,50	104,50
	Total	22		

Test Statistics[a]	
	Smoothness
Mann-Whitney U	38,500
Exact Sig. [2*(1-tailed Sig.)]	,151[b]
a. Grouping Variable: Blind	
b. Not corrected for ties.	

Table 6.5. Mann-Whitney U Test of the rating task for the factor temperature – shows no difference comparing blind and sighted.

Ranks				
	Blind	N	Mean Rank	Sum of Ranks
Temperature	Sighted	11	12,82	141,00
	Blind	11	10,18	112,00
	Total	22		

Test Statistics[a]	
	Temperature
Mann-Whitney U	46,000
Exact Sig. [2*(1-tailed Sig.)]	,365[b]
a. Grouping Variable: Blind	
b. Not corrected for ties.	

Table 6.6. Mann-Whitney U Test of the rating task for the factor slipperiness – shows no difference comparing blind and sighted.

Ranks				
	Blind	N	Mean Rank	Sum of Ranks

Sliperiness	Sighted	11	11,64	128,00
	Blind	11	11,36	125,00
	Total	22		

Test Statistics[a]

	Sliperiness
Mann-Whitney U	59,000
Exact Sig. [2*(1-tailed Sig.)]	,949[b]

a. Grouping Variable: Blind

b. Not corrected for ties.

Table 6.7. Mann-Whitney U Test of the rating task for the factor stiffness – shows no difference comparing blind and sighted.

Ranks

	Blind	N	Mean Rank	Sum of Ranks
Stifness	Sighted	11	10,23	112,50
	Blind	11	12,77	140,50
	Total	22		

Test Statistics[a]

	Stifness
Mann-Whitney U	46,500
Exact Sig. [2*(1-tailed Sig.)]	,365[b]

a. Grouping Variable: Blind

b. Not corrected for ties.

Table 6.8. Mean score of certainty in the memory task analyzed with Mann-Whitney Test – showing no difference comparing blind and sighted.

Ranks

	Blind	N	Mean Rank	Sum of Ranks
Mean_certainty	Sighted	11	11,95	131,50

	Blind	11	11,05	121,50
	Total	22		

Test Statistics[a]	
	Mean_certainty
Mann-Whitney U	55,500
Exact Sig. [2*(1-tailed Sig.)]	,748[b]
a. Grouping Variable: Blind	
b. Not corrected for ties.	

Table 6.9. Crosstab of each material 41, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		,090
N of Valid Cases	22	

Table 6.10. Crosstab of each material 34, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

BIChi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		,670
N of Valid Cases	22	

Table 6.11. Crosstab of each material 13, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Blind * Mat_13

Crosstab		
Count		
	Mat_13	Total

		,00	1,00	
Blind	Sighted	7	4	11
	Blind	2	9	11
Total		9	13	22

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		,080
N of Valid Cases	22	

Table 6.12. Crosstab of each material 16, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Blind * Mat_16

Crosstab				
Count				
		Mat_16		Total
		,00	1,00	
Blind	Sighted	4	7	11
	Blind	1	10	11
Total		5	17	22

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		,311
N of Valid Cases	22	

Table 6.13. Crosstab of each material 2, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Blind * Mat_02

Crosstab
Count

		Mat_02		Total
		,00	1,00	
Blind	Sighted	5	6	11
	Blind	1	10	11
Total		6	16	22

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		,149
N of Valid Cases	22	

Table 6.14. Crosstab of each material 18, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Blind * Mat_18

Crosstab				
Count				
		Mat_18		Total
		,00	1,00	
Blind	Sighted	6	5	11
	Blind	6	5	11
Total		12	10	22

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		1,000
N of Valid Cases	22	

Table 6.15. Crosstab of each material 37, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Blind * Mat_37

Crosstab

Count				
		Mat_37		Total
		,00	1,00	
Blind	Sighted	1	10	11
	Blind	1	10	11
Total		2	20	22

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		1,000
N of Valid Cases	22	

Table 6.16. Crosstab of each material 36, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Blind * Mat_36

Crosstab				
Count				
		Mat_36		Total
		,00	1,00	
Blind	Sighted	6	5	11
	Blind	7	4	11
Total		13	9	22

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test		1,000
N of Valid Cases	22	

Table 6.17. Crosstab of each material 24, comparing blind and sighted with Fisher's Exact Test – showing no significant difference between blind and sighted in the memory task for this material.

Blind * Mat_24

Crosstab

Count

		Mat_24		Total
		,00	1,00	
Blind	Sighted	2	9	11
	Blind	2	9	11
Total		4	18	22

Chi-Square Tests

	Value	Exact Sig. (2-sided)
Fisher's Exact Test		1,000
N of Valid Cases	22	

Table 6.18. Mann-Whitney Test for the factor pleasantness (scale -5 to 5) showing no differences between sighted and blind in any material.

Test Statistics[a]

	Mat_33	Mat_44	Mat_04	Mat_17	Mat_25	Mat_38	Mat_02	Mat_41	Mat_32	Mat_24
Mann-Whitney U	52,500	59,000	49,000	43,500	28,500	41,000	49,000	25,000	51,500	46,000
Exact Sig. [2*(1-tailed Sig.)]	,606[b]	,949[b]	,478[b]	,270[b]	**,034[b]**	,217[b]	,478[b]	**,019[b]**	,562[b]	,365[b]
	Mat_27	Mat_16	Mat_29	Mat_30	Mat_39	Mat_18	Mat_03	Mat_46	Mat_37	Mat_11
Mann-Whitney U	48,500	33,000	42,000	48,000	46,500	36,000	51,000	49,500	59,000	53,000
Exact Sig. [2*(1-tailed Sig.)]	,438[b]	,076[b]	,243[b]	,438[b]	,365[b]	,116[b]	,562[b]	,478[b]	,949[b]	,652[b]

a. Grouping Variable: Blind

b. Not corrected for ties.

Table 6.19. Mann-Whitney Test for the factor smoothness (scale -5 to 5) showing no differences between sighted and blind in any material.

Test Statistics[a]

	Mat_33	Mat_44	Mat_04	Mat_17	Mat_25	Mat_38	Mat_02	Mat_41	Mat_32	Mat_24
Mann-Whitney U	60,000	43,000	41,500	50,000	53,000	45,000	42,000	60,000	49,000	49,500
Exact Sig. [2*(1-tailed Sig.)]	1,000[b]	,270[b]	,217[b]	,519[b]	,652[b]	,332[b]	,243[b]	1,000[b]	,478[b]	,478[b]
	Mat_27	Mat_16	Mat_29	Mat_30	Mat_39	Mat_18	Mat_03	Mat_46	Mat_37	Mat_11
Mann-Whitney U	48,500	53,500	46,000	49,000	45,500	56,500	57,500	27,500	54,000	43,000
Exact Sig. [2*(1-tailed Sig.)]	,438[b]	,652[b]	,365[b]	,478[b]	,332[b]	,797[b]	,847[b]	**,028[b]**	,699[b]	,270[b]

a. Grouping Variable: Blind

b. Not corrected for ties.

Table 6.20. Mann-Whitney Test for the factor temperature (scale -5 to 5) showing no differences between sighted and blind in any material.

Test Statistics[a]

	Mat_33	Mat_44	Mat_04	Mat_17	Mat_25	Mat_38	Mat_02	Mat_41	Mat_32	Mat_24
Mann-Whitney U	41,500	56,500	46,500	43,000	46,500	59,000	39,500	47,000	49,500	51,500
Exact Sig. [2*(1-tailed Sig.)]	,217[b]	,797[b]	,365[b]	,270[b]	,365[b]	,949[b]	,171[b]	,401[b]	,478[b]	,562[b]
	Mat_27	Mat_16	Mat_29	Mat_30	Mat_39	Mat_18	Mat_03	Mat_46	Mat_37	Mat_11

Mann-Whitney U	55,000	44,000	56,500	51,000	55,500	55,000	50,000	53,000	49,000	46,000
Exact Sig. [2*(1-tailed Sig.)]	,748[b]	,300[b]	,797[b]	,562[b]	,748[b]	,748[b]	,519[b]	,652[b]	,478[b]	,365[b]

a. Grouping Variable: Blind

b. Not corrected for ties.

Table 6.21. Mann-Whitney Test for the factor slipperiness (scale -5 to 5) showing no differences between sighted and blind in any material.

Test Statistics[a]

	Mat_33	Mat_44	Mat_04	Mat_17	Mat_25	Mat_38	Mat_02	Mat_41	Mat_32	Mat_24
Mann-Whitney U	60,000	50,500	55,000	58,000	50,500	49,500	39,500	48,500	53,500	53,000
Exact Sig. [2*(1-tailed Sig.)]	1,000[b]	,519[b]	,748[b]	,898[b]	,519[b]	,478[b]	,171[b]	,438[b]	,652[b]	,652[b]
	Mat_27	Mat_16	Mat_29	Mat_30	Mat_39	Mat_18	Mat_03	Mat_46	Mat_37	Mat_11
Mann-Whitney U	46,000	60,500	58,000	42,500	53,500	32,000	59,000	51,500	59,000	40,000
Exact Sig. [2*(1-tailed Sig.)]	,365[b]	1,000[b]	,898[b]	,243[b]	,652[b]	,065[b]	,949[b]	,562[b]	,949[b]	,193[b]

a. Grouping Variable: Blind

b. Not corrected for ties.

Table 6.22. Mann-Whitney Test for the factor stiffness (scale -5 to 5) showing no differences between sighted and blind in any material.

Test Statistics[a]

	Mat_33	Mat_44	Mat_04	Mat_17	Mat_25	Mat_38	Mat_02	Mat_41	Mat_32	Mat_24
Mann-Whitney U	49,500	29,500	42,500	59,000	48,000	54,000	57,500	57,500	47,000	47,500
Exact Sig. [2*(1-tailed Sig.)]	,478[b]	**,040[b]**	,243[b]	,949[b]	,438[b]	,699[b]	,847[b]	,847[b]	,401[b]	,401[b]
	Mat_27	Mat_16	Mat_29	Mat_30	Mat_39	Mat_18	Mat_03	Mat_46	Mat_37	Mat_11
Mann-Whitney U	59,500	47,000	54,000	57,000	46,500	59,500	37,000	43,500	59,500	55,000
Exact Sig. [2*(1-tailed Sig.)]	,949[b]	,401[b]	,699[b]	,847[b]	,365[b]	,949[b]	,133[b]	,270[b]	,949[b]	,748[b]

a. Grouping Variable: Blind

b. Not corrected for ties.

Table 6.23. Mann-Whitney Test for the hit rate, false identification and d' shows no significance difference between sighted and blind memory task score.

Scores				
	Sight	N	Median score	Total score
Hit_rate	Sighted Controls	11	10,68	117,50
	Blind	11	12,32	135,50
	Total	22		
False_identification	Sighted Controls	11	12,59	138,50
	Blind	11	10,41	114,50
	Total	22		
DPRIME (d')	Sighted Controls	11	10,05	110,50
	Blind	11	12,95	142,50
	Total	22		

Statistical tests			
	Hit_rate	False_identification	DPRIME
U de Mann-Whitney	51,500	48,500	44,500
Sig exata [2*(Sig. de unilateral)]	,562[b]	,438[b]	,300[b]
a. Variável de Agrupamento: Sight			
b. Não corrigido para empates.			